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GROWTH AND INJURIOUS EFFECTS OF CRONARTIUM RIBICOLA CANKERS ON PINUS MONTICOLA[1]

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INTRODUCTION

A knowledge of the rate of canker growth and development constitutes a basic element in the study of *Cronartium ribicola* Fischer on its aecial hosts and is of fundamental importance in determining the age of infection (*4*)[3] on *Pinus monticola* Doug. and in considering the rate of damage. Studies of the growth and injurious effects of cankers have therefore been an essential part of the general investigations of the rust on *P. monticola* as carried out by the Portland branch of the Division of Forest Pathology since the discovery of the disease in the native range of this species in the western part of North America in 1921 (*3*).

In these investigations canker is defined as including all stages in the development of the individual infection, from the appearance of the first recognizable discoloration that marks the first symptoms of the disease in the bark. While hyphae are generally present in the bark before the appearance of discoloration, the limits of the discoloration mark rather definitely the advance of the mycelium (*2, p. 625*). Measurements of the extent of the visible symptoms are therefore sufficient for practical purposes.

METHODS

Cankers mainly in the younger stages were selected for growth measurements so that subsequent development might be followed for a number of years. The extremes of length and width of the discolored area were marked at the beginning of the growing season with white lead,[4] a marking material that was found by Rhoads to be satisfactory in studies of blister rust canker growth on *Pinus strobus* L. in 1918 (*6*). After marking, measurements of the extensions of the discoloration beyond these lines were recorded periodically at the end of the growing season in the fall and at the beginning of general growth activity the following spring. Only cankers with clearly defined limits were considered.

It was recognized that such factors as the formation of new cankers, coalescence of cankers, and gnawing of the infected bark by squirrels would render some of the cankers useless for later measurements. Therefore additional cankers were marked to provide against this loss, and all cankers were labeled at first with only temporary labels.

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[2] In the collection of the data on which this paper is based the writer was assisted by C. N. Partington, junior pathologist, and J. L. Mielke, assistant pathologist, Division of Forest Pathology.

[3] Reference is made by number (italic) to Literature Cited, p. 502.

[4] White Duco was used in some of the later experiments reported here and was found to be a better marking material than white lead.

Those suitable for study were permanently labeled when the first measurements were taken.

Data taken for each of the cankers measured were as follows: Place; date; tree number; height of tree at time of marking and subsequent growth in height; condition of cankers and number on tree; serial number of canker and location on tree (i.e., whether on branch or trunk); diameter below canker, growth rate, and condition of infected branch or trunk; age of internode on which canker started; length and width of cankered area at time of marking; age of canker, as nearly as could be determined; subsequent canker growth upward, downward, to left, and to right; and general notes on growth of cankers, production of fruiting stages, etc. The initial measurements were taken for the greatest lengths and widths of the cankers, and the same principle was followed for all subsequent measurements. In general, the latter fell in line with the first measurements, but frequently an irregular extension threw the lines of measurement off the original axes.

Trees selected in the study were mainly thrifty young specimens ranging between 5 and 25 feet in height. Cankers were marked on twigs, branches, and trunks of all diameters from the smallest to the largest and of all conditions, from thrifty to suppressed.

Growth rates established by these measurements were supplemented by the observed development of the marked cankers and by observational analyses of the extent of growth and of the effect of large numbers of other cankers of all sizes and ages on all classes and conditions of trees throughout a much wider range, thus providing the basis for the conclusions on injurious effects of canker growth.

BASIS OF COMPARATIVE STUDIES OF CANKERS IN VARIOUS AREAS

Although *Cronartium ribicola* is now widely distributed throughout most of the range of *Pinus monticola* in the Pacific Northwest [5] (*4, p. 1*), at the time the growth studies were begun the main range of the disease was centered in southwestern British Columbia. Therefore most of the areas in which measurements were made are located in this section. The areas were selected to represent site and climatic conditions generally favorable to the growth of *P. monticola* and at the same time to provide a range in these conditions that would give a measure of the effect of site and climate upon canker growth. Accordingly some of the areas are situated near sea level, where the growing season is relatively long, and others are located at higher elevations and more inland points, where the season is materially shorter. All are in British Columbia.

The following areas were selected: Chee Kye, near sea level on the Cheakamus River about 50 miles north of Vancouver; Bold Point, on Quadra Island, and Thurston Bay, on Sonora Island, both at sea level about 110 and 130 miles, respectively, northwest of Vancouver; Daisy Lake, at an elevation of 1,300 feet in the Coast Range about 60 miles north of Vancouver; Mile 72, on the Pacific Great Eastern Railway, at an elevation of 1,300 feet in the Coast Range about 110 miles north of Vancouver; Revelstoke, at an elevation of 1,500 feet on the Columbia River about 250 miles east of Vancouver; and a

[5] Pacific Northwest as used in this paper includes southern British Columbia, Washington, Oregon, Idaho, and western Montana.

locality near Arrow Park, at an elevation of 1,700 feet on the Arrow Lakes about 60 miles south of Revelstoke. At the first three places growth rates of *Pinus monticola* are closely comparable to those in the optimum range of the species in northern Idaho and western Montana. At the last four places the growing season is normally 1 month to 1½ months shorter than at the first three places, and growth is generally slower.

At the beginning of the growing season in the spring of 1923 about 100 cankers were marked at each of the above-named places except Revelstoke and Arrow Park. The great size of the unopened territory covered in the investigations during this year made it impossible to secure measurements in the fall except at Chee Kye and Thurston Bay. Measurements were made in the spring of 1924, approximately 1 year after the marking, at all places except Mile 72, where the trees had been destroyed in a forest fire which swept that section in the fall of 1923. Trees in the Bold Point area were likewise destroyed by fire in 1924. In the other areas measurements were not attempted after the spring of 1924, because of the death of the cankers or complications of their growth from various causes.

At Revelstoke 74 cankers were marked in the spring of 1928 and measured in the fall. Those that were not complicated by coalescence of cankers, squirrel feeding, etc., were remeasured in the fall of 1929. At Arrow Park 138 cankers were marked in October 1929 and measured at the start of the growing season in the spring of 1930 and again at the end of the growing season in the fall.

A special study of the growth of trunk cankers was made at Chee Kye and Daisy Lake on cankers marked in the fall of 1925. Measurements of these were made at Daisy Lake in the spring of 1926 and the fall of 1926 and of 1927, and at Chee Kye in the fall of 1926 and of 1927.

Because of the complicating factors enumerated, only a part of the cankers that were marked remained suitable for measurements in most of the areas. As already stated, the three localities—Chee Kye, Bold Point, and Thurston Bay—are representative in growth characteristics of the optimum for *Pinus monticola* and are similar in general growth conditions for the rust. The size and condition of the trees and branches on which the cankers were marked were similar in all areas. The trees were thrifty, young, open-grown specimens, and the stems bearing the marked cankers were generally in thrifty or good condition. As was to be expected, therefore, the individual growth performances and general growth averages of the cankers at these places were generally comparable. In view of the general similarity of these areas, the values for these three are capable of combination.

The measurements made at the same time at Daisy Lake showed a much slower growth of cankers than in the three other areas. Canker growth was likewise slower at Revelstoke and Arrow Park. But while the general site conditions for canker growth of these three areas are fairly comparable, differences in the character of the cankers and of the trees and branches on which they occurred make the values from them unsuitable for combination, and therefore each must be considered separately.

At Daisy Lake the marked cankers were located primarily on the smaller branches that were in only fair or in poor condition, in the

lower crowns of rather large trees, or on suppressed smaller trees. At Revelstoke only branch cankers were marked. In general, the cankers were older at the time of marking than were those at the other places. The affected branches at Revelstoke soon entered a stage of such rapid decline that most of them died from the canker outward to the tip during the following season. The cankers marked at Arrow Park were all branch cankers. In general, these cankers and the trees and branches on which they occurred were comparable in size and relative thrift with those in the optimum areas. They offer a good opportunity, therefore, for comparison with those of the optimum areas for canker growth under contrasting climatic and site conditions. Further comparison of growth under such conditions is available primarily from the special study of trunk cankers at Daisy Lake and Chee Kye from 1925 to 1927.

GROWTH OF CANKERS

RATE OF GROWTH IN RELATION TO SIZE AND VIGOR OF STEM

At the beginning of the investigations it was evident from general observations of cankers of similar age that the rate of canker growth varied decidedly with the size and, to some degree, with the vigor of the affected part. Rhoads, on the basis of growth measurements of the rust on *Pinus strobus* in 1918, stated (*6, p. 517*): "In general it may be said that the growth of the blister rust cankers is in direct proportion to the size and vigor of the stems on which they occur." He offered no concrete evidence, however, on the influence of vigor, and in his table 1 he has combined branch, twig, and trunk cankers without reference to vigor. What part vigor plays in the values he has shown for correlation with size is therefore uncertain. From the present study it appears that the factor of size, corresponding with the amount of bark tissue available, entirely predominates over that of vigor; therefore, Rhoads' values are probably fairly indicative of the size factor.

In studying the influence of vigor on cankers it was thought best in the present work to consider twig and branch cankers separately from trunk cankers, since the growth of the trunks and leaders is normally more rapid and vigorous than that of the branches. In the classification of trunks and leaders, as compared with branches and twigs in *Pinus monticola*, a further distinction is naturally suggested by the characteristic differences in their general habit of growth. The trunks are normally straight and erect, whereas the branches are horizontal and more or less curved; the internodes of the trunks are longer and taper more slowly than those of the branches and the nodes of the trunks are distinctly marked by the regular radially whorled arrangement of the branches, whereas on the branches the habit of branching is flatter and more irregular and the nodes are less distinctly marked. Normally, a trunk of given diameter will be considerably younger and more active in growth than a branch of equal size.

Comparisons were made of canker growth in the same place and at the same season on trunks and branches of equal size. Provided the condition of the branches and trunks was fair or better, no essential differences between them in growth rate of cankers were evident; therefore, the more rapid growth and greater vigor of the trunks was

without apparent influence. In this connection it should be stated that the bark, which is available for mycelial growth, is slightly thicker on the branches than on the trunks, which are younger though of equal size. Conditions for canker growth, therefore, were a little more favorable on the branches than on the trunks. On the other hand, if this slight difference in bark thickness offsets the difference in vigor, it would appear that the influence of the latter was, at the most, comparatively slight. It was evident, therefore, that vigor or lack of vigor would have to be extreme in order to have a material influence upon the growth of the fungus.

On the other hand, the bulk of the living bark tissues, as indicated by the size of the part infected, was clearly the most essential condition of the host for rapid growth of the canker.

ANNUAL LONGITUDINAL GROWTH

Influence of Size of Stems

The influence of the size of the part infected on canker growth is shown in table 1, which gives, by diameter classes of the part infected, the average annual longitudinal growth of branch and trunk cankers at Chee Kye and Thurston Bay and of branch cankers at Bold Point, measured in the spring of 1924. Growth rates at Chee Kye and Thurston Bay were so nearly identical that the data for these areas are combined. Canker growth at Bold Point was somewhat more rapid, and since the measurements here consisted almost entirely of branch cankers, the values for this area are given separately.

TABLE 1.—*Average longitudinal growth, April 1923 to April 1924, of branch and trunk cankers at Chee Kye and Thurston Bay and branch cankers at Bold Point, British Columbia*

Stem-diameter class (inches)	Chee Kye and Thurston Bay						Bold Point		
	Branch cankers			Trunk cankers			Branch cankers [a]		
	Cankers	Average branch diameter	Canker growth	Cankers	Average trunk diameter	Canker growth	Cankers	Average branch diameter	Canker growth
	Number	*Inches*	*Inches*	*Number*	*Inches*	*Inches*	*Number*	*Inches*	*Inches*
0.1									
0.2–0.3	20	0.28	4.12				10	0.28	3.93
0.4–0.5	21	.43	4.50	1	0.50	4.90	15	.47	5.13
0.6–1.0	6	.62	5.63	3	.83	5.60	27	.73	6.39
1.1–1.5				4	1.33	6.20	5	1.32	7.20
1.6–2.0				8	1.76	7.50			
2.1–2.5									
2.6–3.0				2	2.90	8.45			
3.1–3.5									

[a] In addition, measurements were secured on 1 trunk canker, stem diameter 2.5 inches, canker growth 8.6 inches.

The increase in the rate of canker growth with size of the part infected is striking. The curve of growth rate over size of part infected is parabolic in form. This is indicated in figure 1, in which the Chee Kye and Thurston Bay data of table 1 are plotted. The short curve for the growth of branch cankers is so close to the beginning of the much more extended curve for the growth of trunk cankers

that for all practical purposes it may be regarded as an extension of the longer curve. Under the conditions found at Chee Kye and Thurston Bay, from April 1923 to April 1924, which may be considered fairly representative of the optimum, the growth rate of cankers increases very rapidly for the smaller stem-diameter classes, attaining an average of 6 to 8 inches a year for stem diameters from 1 to 2 inches.

For growth rates at still larger diameters under these conditions, it is necessary to consider the measurements of 1926 of the trunk cankers from the Chee Kye area. The year from October 1925 to October 1926 covered by these measurements, was comparable in seasonal conditions for canker growth to the year from April 1923 to April 1924, covered by the branch- and trunk-canker measurements at Chee Kye and Thurston Bay. The condition of the cankers and the class and condition of the trees on which they occurred were likewise comparable for the two sets of measurements. The 1926 data at Chee Kye shown in table 2 therefore constitute a natural extension of the 1924 data given in table 1.

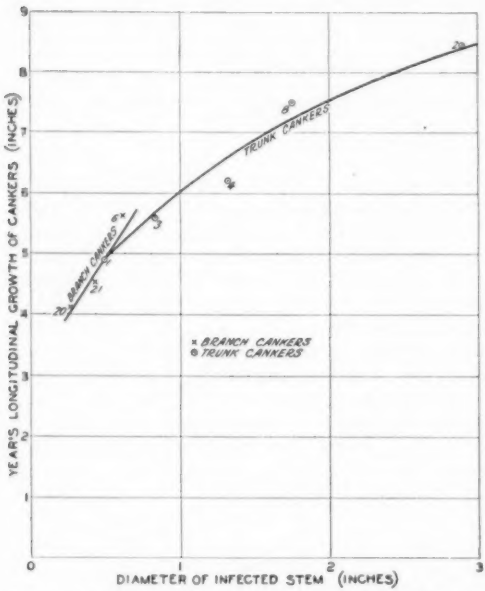

FIGURE 1.—Average longitudinal growth from April 1923 to April 1924 of cankers marked at Chee Kye and Thurston Bay, British Columbia, in April 1923.

From table 2 it is evident that the increase in growth rate of cankers tapers off very rapidly in the larger stem-diameter classes. This tendency and that of the 1926 Chee Kye data to coincide with and extend the 1924 Chee Kye and Thurston Bay values are still more evident from figure 2, in which the curve of the 1926 Chee Kye values is given together with the curves of the 1924 Chee Kye and Thurston Bay data.

TABLE 2.—*Average longitudinal growth from Oct. 31, 1925, to Oct. 16, 1926, of trunk cankers marked near Chee Kye, British Columbia, Oct. 31, 1925*

Trunk-diameter class (inches)	Cankers	Average trunk diameter	Canker growth	Trunk-diameter class (inches)	Cankers	Average trunk diameter	Canker growth
	Number	*Inches*	*Inches*		*Number*	*Inches*	*Inches*
2.6–3.0	2	2.85	8.15	6.1–7.0	3	6.47	8.83
3.1–4.0	10	3.56	8.98	7.1–8.0	1	7.90	9.10
4.1–5.0	6	4.60	10.15	11.1–12.0	1	11.80	9.50
5.1–6.0	3	5.33	8.37				

Beyond the 2-inch stem-diameter class the longitudinal growth rate of cankers increases very much more slowly, and beyond the 4-inch or 5-inch stem-diameter class it tends to become stabilized or constant. In the data under consideration, representing optimum conditions, the maximum or constant rate of canker growth averages between 9 and 10 inches a year.

Influence of Vigor of Stems

The relative condition of the branches and trunks on which the cankers forming the basis for tables 1 and 2 and figures 1 and 2 occurred was characterized as poor, fair, good, and thrifty. In the main, the condition was either good or thrifty. Since no differences in growth rate attributable to differences in thrift were evident between branch cankers and trunk cankers, an effort was made to

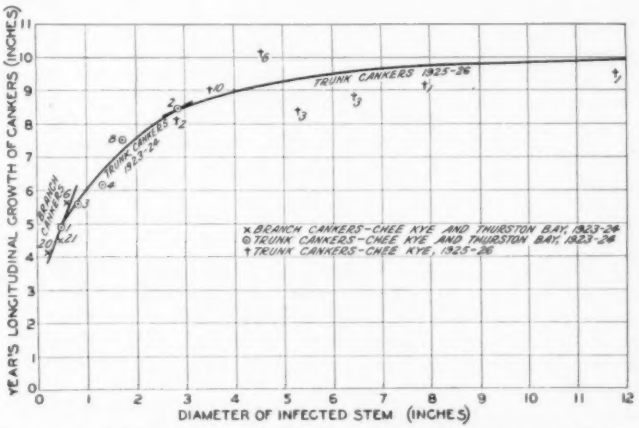
YEAR'S LONGITUDINAL GROWTH OF CANKERS (INCHES)
DIAMETER OF INFECTED STEM (INCHES)
BRANCH CANKERS
TRUNK CANKERS 1923-24
TRUNK CANKERS 1925-26
× BRANCH CANKERS - CHEE KYE AND THURSTON BAY, 1923-24
⊙ TRUNK CANKERS - CHEE KYE AND THURSTON BAY, 1923-24
† TRUNK CANKERS - CHEE KYE, 1925-26

Figure 2.—Longitudinal growth rates of *Cronartium ribicola* on *Pinus monticola* under optimum conditions at Chee Kye and Thurston Bay, British Columbia.

establish as far as possible the extent of the influence of this factor by comparison of these cankers according to the vigor classification of the trunks or branches. These classifications were based on the growth rate and apparent condition of the branch or trunk during the year from time of marking to time of measurement. Each canker occurring on a stem that at the time of measurement was classified as poor or fair was paired with the nearest listed canker of most nearly comparable stem diameter, in the same area and year of measurement, occurring on a stem classified as thrifty. No canker was used twice. In case a canker in the thrifty-stem classification was not available for comparison, the nearest canker occurring on a stem classified as good was used. The longitudinal growth of these mathematically paired cankers is compared in table 3. The values do not indicate that differences in the vigor of the stems had any influence on the growth rates of the cankers. The averages are practically identical. The only decided influence is that of size of the part infected.

TABLE 3.—*Comparison of longitudinal growth of cankers on stems in poor or fair condition with that of cankers on stems of similar size but in thrifty or good condition at Chee Kye and Thurston Bay, British Columbia*

Pair of cankers compared (no.)	Stem in poor or fair condition				Stem in thrifty or good condition			
	Stem diameter	Condition of stem [a]		Canker growth	Stem diameter	Condition of stem [a]		Canker growth
		At marking	At measuring			At marking	At measuring	
	Inches			*Inches*	*Inches*			*Inches*
1	0.2	F	P	4.0	0.2	T	G	4.4
2	.2	F	F	4.8	.3	G	G	4.8
3	.3	P	P	4.3	.3	T	T	4.3
4	.3	F	P	4.1	.3	G	G	3.6
5	.3	F	F	3.4	.3	G	G	4.4
6	.4	F	F	4.5	.4	G	G	4.7
7	.4	G	P	3.7	.4	G	G	3.9
8	.5	F	F	6.1	.4	T	G	4.7
9	3.0	T	P	7.6	2.7	T	T	8.7
10	3.2	G	F	9.9	3.2	T	T	8.5
11	3.4	G	F	8.0	3.4	G	T	10.0
12	3.5	T	F	8.6	3.6	T	T	8.9
13	4.0	G	F	9.3	4.0	T	T	9.1
14	4.6	G	F	9.8	4.6	T	T	9.0
15	5.0	G	F	10.6	5.2	T	T	7.7
16	5.5	P	P	8.8	5.3	T	T	8.6
17	6.4	T	F	9.1	6.8	T	T	8.3
Total	41.2	3 T, 6 G, 6 F, 2 P	11 F, 6 P	116.6	41.4	11 T, 6 G	10 T, 7 G	113.6
Average	2.42	G–F	F–P	6.86	2.44	T–G	T–G	6.68

[a] P=poor; F=fair; G=good; T=thrifty.

Evidently, under ordinary conditions these vigor classifications do not sufficiently segregate the extremes in vigor and weakness necessary to exert any marked influence on canker growth. It was observed, however, that great vigor, as in the case of young, very fast-growing leaders and shoots, did have a noticeably stimulating effect on the rate of canker growth.

Moreover, it was evident from the data from Revelstoke that extreme lack of vigor exerts a decidedly retarding influence on canker growth. The cankers at Revelstoke were marked in April 1928 and measured the last of September 1928 and again at the beginning of October 1929. During the growing season of 1928, covered by the first measurements, the branches on which the cankers occurred were generally in fair condition. During 1929 a large number of the branches were partially or completely killed by the cankers, and the vigor of the remaining branches was greatly reduced. On these latter branches, the average diameter of which was 0.44 inch, the average longitudinal growth for 24 cankers was 2.99 inches from April 22 to September 23, 1928, and 3.14 inches from September 23, 1928, to October 8, 1929.

These values show that the growth for the growing season from April to September 1928, during which the affected branches were in relatively fair condition, almost equaled the growth for the entire year from September 1928 to October 1929. Naturally, growth

during the winter or dormant season is much slower than during the growing season. Nevertheless, it represents a considerable fraction of the total annual growth. This aspect will be discussed in the section on winter growth. It is evident, therefore, that the growth of these cankers for the growing season of 1929, during which the vigor of the affected branches was nearly exhausted, must have been considerably below that for the growing season of 1928. There were no differences in climatic conditions between the two seasons to cause this difference in growth. Therefore, the reduced growth rate of the cankers during 1929 can be explained only on the basis of their extremely reduced vigor in that year. The measurements at Revelstoke afforded the only concrete evidence secured in the present study that vigor had any material effect on the growth of the cankers.

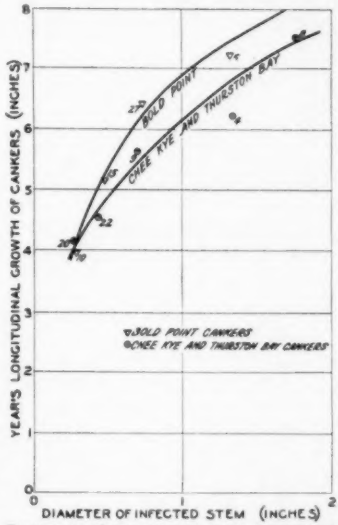
YEAR'S LONGITUDINAL GROWTH OF CANKERS (INCHES)
DIAMETER OF INFECTED STEM (INCHES)
BOLD POINT
CHEE KYE AND THURSTON BAY
●BOLD POINT CANKERS
○CHEE KYE AND THURSTON BAY CANKERS

FIGURE 3.—Comparison of year's longitudinal growth of cankers at Chee Kye and Thurston Bay with cankers at Bold Point, for the period from April 1923 to April 1924.

Influence of Site and Climate

There seems to be a close correlation between regional site conditions and the rate of canker growth. Even when seasonal climatic differences within a given region are slight, the differences in canker growth are frequently marked. During the year 1923–24 the seasonal differences at Bold Point, Chee Kye, and Thurston Bay were too insignificant to become apparent through general observations. All these points lie at low elevations close to the Strait of Georgia and are within the same climatic belt. However, a comparison of canker growth at Bold Point with that at Chee Kye and at Thurston Bay (table 1 and fig. 3) shows that in general the growth of the Bold Point cankers averaged almost one half inch more for the year than that of the cankers in similar stem-diameter classes at Chee Kye and Thurston Bay.

Where the difference in climatic and environmental conditions is greater, the difference in canker-growth rates is striking. This is shown by comparing the growth averages of the cankers measured at Daisy Lake, which has a shorter growing season (table 4), with those of the cankers measured during the same periods at Chee Kye and Thurston Bay (tables 1 and 2). In figure 4 these data are compared graphically.

TABLE 4.—*Average longitudinal growth of branch cankers from April 1923 to April 1924 and of trunk cankers from October 1925 to October 1926, at Daisy Lake, British Columbia*

Stem-diameter class (inches)	Branch cankers marked Apr. 10–16, 1923; measured Apr. 19, 1924			Trunk cankers marked Oct. 31, 1925; measured Oct. 16, 1926		
	Number	Average branch diameter	Canker growth	Number	Average trunk diameter	Canker growth
		Inches	*Inches*		*Inches*	*Inches*
0.1	38	0.134	1.83			
0.2–0.3	37	.216	2.28			
0.4–0.5	1	.500	3.70			
0.6–1.0				[a] 2	0.70	4.25
1.1–1.5				1	1.20	4.40
1.6–2.0				2	1.75	5.35
2.1–2.5				2	2.30	5.55
2.6–3.0				6	2.83	5.60
3.1–4.0				12	3.68	5.93
4.1–5.0				6	4.77	6.85
5.1–6.0				2	5.75	6.80
6.1–7.0				1	6.10	6.60

[a] Marked April 1923; measured April 1924.

Although the growth rates at Daisy Lake were uniformly much below those in the optimum areas, figure 4 shows a similarity in the

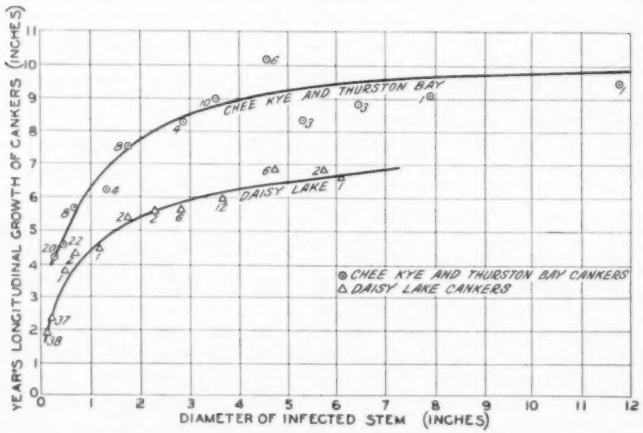
YEAR'S LONGITUDINAL GROWTH OF CANKERS (INCHES)
DIAMETER OF INFECTED STEM (INCHES)
CHEE KYE AND THURSTON BAY
DAISY LAKE
CHEE KYE AND THURSTON BAY CANKERS
DAISY LAKE CANKERS

FIGURE 4.—Comparison of year's longitudinal canker-growth rates at Daisy Lake with those at Chee Kye and Thurston Bay.

general form of the curves for the two sets of data. Under conditions represented at Daisy Lake, canker growth increased rapidly from a rate of less than 2 inches per year for cankers in the 0.1-inch stem-diameter class to over 5 inches per year for those with stem diameters of between 1.6 and 2.0 inches. Beyond such diameters the curve of growth rapidly levels off and attains a maximum average of between 6 and 7 inches per year at stem diameters beyond 4 or 5 inches. In

the optimum areas, on the other hand, the rate increases rapidly from about 4 inches per year in the 0.2- to 0.3-inch stem-diameter class to about 8 inches per year at a stem diameter of about 2 inches. Beyond diameters of 4 or 5 inches the curve of growth levels off to a maximum of between 9 and 10 inches per year.

The similarity in the general climatic conditions of Revelstoke, Arrow Park, and Daisy Lake results in a similarity of canker growth at all three stations. But since only branch cankers were measured at Revelstoke and Arrow Park, no comparison is available in the larger stem-diameter classes; and, as stated previously, the cankers measured at Revelstoke were older than those measured elsewhere and the branches on which they occurred were exceptionally low in vigor, whereas the cankers measured at Arrow Park and the branches on which they occurred were decidedly thrifty; the branches on which cankers were measured at Daisy Lake, on the other hand, were generally in only fair condition. Furthermore, a different annual-growth period was covered at each of the three places. Consequently, the relationships between these areas are somewhat obscure. Nevertheless they afford some significant comparisons with the other data.

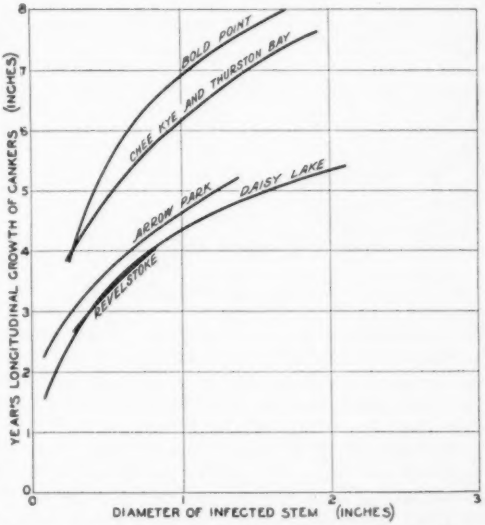

FIGURE 5.—Comparison of year's longitudinal canker-growth rates in all areas.

The longitudinal growth values for the cankers of Revelstoke, Arrow Park, Daisy Lake, Chee Kye and Thurston Bay, and Bold Point are given in table 5. Figure 5 gives the growth curves for all the areas, covering the diameter classes in which all may be compared. The relative thrift conditions of the branches at Arrow Park were entirely comparable with those of Chee Kye and Thurston Bay and those of Bold Point, that is, the branches in each area were generally thrifty for the site conditions represented. The values and curves for Arrow Park, therefore, may be compared with those of the optimum areas to show further evidence of the influence of climate and general site conditions on canker growth. This comparison shows that despite the highly vigorous condition of the affected branches, growth—as in the case of the cankers at Daisy Lake—is decidedly slower than in the optimum areas.

TABLE 5.—*Average annual longitudinal growth of marked cankers in various localities*

Stem-diameter class (inches)	Revelstoke (Sept. 23, 1928, to Oct. 8, 1929)			Arrow Park (Oct. 11, 1929, to Sept. 21, 1930)			Daisy Lake			Chee Kye and Thurston Bay			Bold Point (April 1923 to April 1924)		
	Cankers	Average stem diameter	Canker growth	Cankers	Average stem diameter	Canker growth	Cankers	Average stem diameter	Canker growth	Cankers	Average stem diameter	Canker growth	Cankers	Average stem diameter	Canker growth
	No.	*In.*	*In.*	*No.*	*In.*	*In.*	*No.*	*In.*	*In.*	*No.*	*In.*	*In.*	*No.*	*In.*	*In.*
0.1				7	0.10	2.41	[a]38	0.134	1.83						
0.2–0.3	7	0.29	2.91	112	.23	2.81	[a]37	.216	2.28	[b]20	0.27	4.12	10	0.28	3.93
0.4–0.5	13	.44	2.97	16	.44	3.49	[c]1	.50	3.70	[b]22	.43	4.52	15	.47	5.13
0.6–1.0	4	.73	4.08	2	.65	3.50	[c]2	.70	4.25	[b]9	.69	5.62	27	.73	6.39
1.1–1.5				1	1.20	5.70	[c]1	1.20	4.40	[b]4	1.33	6.20	5	1.32	7.20
1.6–2.0							[c]2	1.75	5.35	[b]8	1.76	7.50			
2.1–2.5							[c]2	2.30	5.55						
2.6–3.0							[c]6	2.83	5.60	[b]2 [d]2	2.88	8.30			
3.1–4.0							[c]12	3.68	5.93	[d]10	3.56	8.98			
4.1–5.0							[c]6	4.77	6.85	[d]6	4.60	10.15			
5.1–6.0							[c]2	5.75	6.80	[d]3	5.33	8.37			
6.1–7.0							[c]1	6.10	6.60	[d]3	6.47	8.83			
7.1–8.0										[d]1	7.90	9.10			
11.1–12.0										[d]1	11.80	9.50			

[a] April 1923 to April 1924.
[b] Chee Kye and Thurston Bay (April 1923 to April 1924).
[c] October 1925 to October 1926.
[d] Chee Kye (October 1925 to October 1926).

In connection with the general application of the results reported here the difference shown between the Arrow Park values and those of the optimum areas is important. The Arrow Park area is situated on the western edge of the northward extension of the main commercial range of *Pinus monticola* in the "Inland Empire" section of northern Idaho and adjacent Washington and Montana. The general site and climatic conditions for *P. monticola* in this area are more typical of the main range than are those in the optimum areas.[6] In the main range of the pine, therefore, the rates of canker growth may be expected to fall between that at Arrow Park and the optimum and to be probably somewhat closer to the former than to the latter.

The growth rates at Revelstoke (fig. 5) are among the slowest shown. In this case, however, as has already been shown, the growth of the cankers was materially retarded by the extreme lack of vigor of the affected branches.

WINTER GROWTH

As previously stated, canker growth during the winter, or dormant season, is much slower than during the growing season. In the present studies the growing season is defined as the 6 months following the beginning of general growth activity in the spring, and the winter season as the remaining 6 months of the year. Measurements of the

[6] The fact that the main commercial range does not include all optimum growth of *Pinus monticola* will undoubtedly seem strange. The reason for this appears primarily to be that in the coastal region of the range of the pine—that is, in the country surrounding the Strait of Georgia and southward, which presents optimum conditions for this species and includes the optimum areas of the present study—the conditions are particularly favorable for its principal bark beetle, *Dendroctonus monticolae* Hopk. Between diameters, at breast height, of about 10 to 20 inches, the pine appears to be especially susceptible to the attacks of this beetle which decimates the stand to a very small and scattered representation at maturity. This has been a common observation of western entomologists. The relationship was first pointed out to the writer in 1926 by Ralph Hopping, Dominion entomologist, at Vernon, British Columbia.

winter growth of cankers were secured at Chee Kye and Thurston Bay in 1924, at Daisy Lake in 1926, and at Arrow Park in 1930. These data, together with those on annual growth for the same cankers are given in table 6. The periods covered in the measurements of winter growth were October 8, 1923, to April 17, 1924, at Chee Kye; October 19, 1923, to April 8, 1924, at Thurston Bay; October 31, 1925, to April 15, 1926, at Daisy Lake; and October 9 to 11, 1929, to May 6, 1930, at Arrow Park.

TABLE 6.—*Average annual and winter longitudinal growth of cankers at various localities*

Stem-diameter class (inches)	Chee Kye and Thurston Bay					Daisy Lake					Arrow Park				
	Cankers	Average stem diameter	Canker growth			Cankers	Average stem diameter	Canker growth			Cankers	Average stem diameter	Canker growth		
			Annual	Winter				Annual	Winter				Annual	Winter	
	No.	*In.*	*In.*	*In.*	*Pct.*[a]	*No.*	*In.*	*In.*	*In.*	*Pct.*[a]	*No.*	*In.*	*In.*	*In.*	*Pct.*[a]
0.1											7	0.10	2.41	0.40	16.6
0.2–0.3	20	0.28	4.12	0.68	16.5						112	.23	2.81	.46	16.4
0.4–0.5	[b]21	.43	4.50	.84	18.7						16	.44	3.49	.73	20.9
0.6–1.0	9	.69	5.62	.94	16.7						2	.65	3.50	.55	15.7
1.1–1.5	4	1.33	6.20	.98	15.8	1	1.20	4.40	0.9	20.5	1	1.20	5.70	1.30	22.8
1.6–2.0	8	1.76	7.50	1.08	14.4	2	1.75	5.35	1.0	18.7					
2.1–2.5						2	2.30	5.55	.8	14.4					
2.6–3.0	2	2.90	8.45	1.20	14.2	6	2.83	5.60	.65	11.6					
3.1–4.0						12	3.68	5.93	.90	15.2					
4.1–5.0						5	4.76	6.42	1.22	19.0					
5.1–6.0						2	5.75	6.80	.85	12.5					
6.1–7.0						1	6.10	6.60	.60	9.1					
Total or av.	64	.72	5.14	.85	16.5	31	3.61	5.91	.89	15.1	138	.26	2.90	.50	17.2

[a] Winter growth expressed as percentage of annual growth.
[b] 1 canker given in table 5 not included for lack of separate winter growth measurements.

The winter growth averages about 15 to 17 percent of the annual growth, and about 18 to 20 percent, or about one sixth to one fifth, of that made during the growing season. There was considerable variation in the winter and the summer growth of individual cankers, although the total or annual growth values in these cases were fairly close to the averages. This fact indicates a rather wide variation in the periods of main growth activity for individual cankers, as was brought out by Rhoads' studies in *Pinus strobus* in 1918 (*6, p. 517*).

UPWARD AND DOWNWARD GROWTH

Throughout the present studies, measurements were taken of the longitudinal extensions of the cankers in both directions, that is, upward, or against the sap stream in the bark of the branch or trunk, and downward, or with the sap stream. The measurements generally showed a greater growth downward than upward. The same tendency was noted on *Pinus strobus* by Rhoads in 1918 (*6, p. 516*). Downward and upward growth averages for the main canker groups in the present study are given in table 7.

TABLE 7.—*Average annual downward and upward growth of cankers in various localities*

Locality	Type of cankers	Year of measurement	Cankers	Average stem diameter	Canker growth				
					Average total	Average upward	Downward		
							Average	Proportion of total growth	Superiority over upward growth
			Number	*Inches*	*Inches*	*Inches*	*Inches*	*Percent*	*Percent*
Bold Point	Branch	1924	57	0.63	5.70	2.71	2.98	52.4	10.0
Chee Kye and Thurston Bay.	do	1924	47	.39	4.48	2.05	2.43	54.3	18.9
Do	Trunk	1924	19	1.67	6.88	3.31	3.57	51.9	7.9
Daisy Lake	Branch	1924	76	.18	2.08	.80	1.19	57.2	33.6
Do	Trunk	1926	32	3.64	6.01	3.03	2.98	49.5	* 2.0
Chee Kye	do	1926	26	4.77	8.97	4.48	4.49	50.0	.2
Revelstoke	Branch	1929	24	.44	3.14	1.28	1.85	59.1	44.5
Arrow Park	do	1930	138	.26	2.90	1.37	1.53	52.8	12.1
Total			419						

* Inferiority.

The superiority of downward growth over upward growth appears to be very largely due to the size of the part infected, since downward growth runs into greater stem diameters, whereas upward growth runs into smaller diameters. As might be expected on this basis, the difference between downward and upward growth is generally greater on the twigs and branches than on the trunks, since relative taper is normally more rapid in the twigs and branches than in the trunks. Furthermore, the influence of the difference in diameters at the lower and upper ends of the canker is accentuated in the branches because of the fact that the branches fall in the smaller diameter classes where differences in stem diameter have the greatest effect on canker growth. The difference between downward and upward growth is particularly marked in the case of the Daisy Lake branch cankers, where growth of the affected branches was slower and taper was more rapid than normally. The difference was still more marked in the Revelstoke branch cankers, which, being older and therefore relatively longer than the average, extended over greater lengths of the branches and thereby increased the differences in stem diameter at the lower and upper ends of the cankers, from which growth was measured.

In addition to the influence of differences in diameter, the superiority in downward growth may be partly accounted for on the hypothesis that downward growth of the mycelium is aided by the downward flow of sap in the bark. There was, however, no means of checking such an assumption.

While downward growth was generally greater than upward growth, there were frequent individual exceptions to this rule, and sometimes upward growth was very decidedly greater than downward growth. It was frequently observed that longitudinal growth in both directions was checked for a time at the nodes, particularly where branching was heavy and the nodes were enlarged and swollen. In most cases where the upward growth was the greater, the downward growth had

been retarded from this cause. In some cases, however, where such an explanation was not applicable, the cause of the excess in upward growth could not be determined.

LATERAL OR GIRDLING GROWTH

Equally as important as longitudinal growth, or even more important, is the growth of the canker laterally, since it is through growth in this direction that the stem is finally girdled and killed. Measurements on such growth were secured wherever possible in all cankers studied. In the case of the cankers which were marked on the branches, the infection had generally completely surrounded the stem at the time of marking. Therefore trunk cankers, and mainly the larger ones, provided the chief basis for measurements of lateral growth. The data secured for annual growth in this direction were practically confined to the 1926 trunk-canker measurements at Chee Kye and Thurston Bay and at Daisy Lake. Table 8 gives annual longitudinal and lateral growth averages of all cankers on which complete measurements of both longitudinal and lateral growth were secured. No difference was noted, or expected, in the average rates of extension to the right and left, and the values are therefore combined in table 8.

TABLE 8.—*Average annual growth of cankers at Chee Kye and Daisy Lake, British Columbia*

Stem-diameter class (inches)	Chee Kye, Oct. 20–21, 1925, to Oct. 13–14, 1926					Daisy Lake, Oct. 31, 1925, to Oct. 16, 1926				
	Cankers	Average stem diameter	Canker growth			Cankers	Average stem diameter	Canker growth		
			Longitudinal	Lateral				Longitudinal	Lateral	
	Number	*Inches*	*Inches*	*Inches*	*Percent* [a]	*Number*	*Inches*	*Inches*	*Inches*	*Percent* [a]
1.6–2.0						1	1.60	5.50	2.00	36.4
2.1–2.5						2	2.30	5.55	2.15	38.7
2.6–3.0	2	2.85	8.15	2.50	30.7	5	2.84	5.54	2.52	45.5
3.1–4.0	9	3.60	8.88	3.21	36.1	12	3.68	5.93	2.61	44.0
4.1–5.0	4	4.60	9.45	3.48	36.8	6	4.77	6.85	2.55	37.2
5.1–6.0	3	5.33	8.37	3.67	43.8	2	5.75	6.80	2.65	39.0
6.1–7.0	2	6.60	8.70	3.65	42.0	1	6.10	6.60	2.40	36.4
11.1–12.0	1	11.80	9.50	3.70	38.5					
Total or average	21	4.64	8.86	3.33	37.6	29	3.82	6.10	2.52	41.3

[a] Lateral growth expressed as percentage of longitudinal growth.

The data in table 8 show that lateral growth is decidedly slower than longitudinal growth. In general, it amounts to about 40 percent of the longitudinal growth. In these proportions lateral growth appears to show about the same variations as longitudinal growth with respect to all the influences affecting the latter.

One rather singular exception to the general relations between lateral and longitudinal growth is exhibited in the earliest stages of the young canker, particularly on the larger stems. For perhaps the first 2 or 3 weeks following the appearance of the canker, the discoloration advances so much more rapidly laterally than longitudinally that in this stage the initial discoloration assumes an oval shape, having the long diameter at right angles to the longitudinal axis of

the stem. Later the longitudinal growth is accelerated to its normal rate and the discoloration assumes its characteristic shape, that is, an oval having the long diameter parallel with the longitudinal axis of the stem. This phenomenon would be an interesting subject for physiological study.

The foregoing data have dealt almost entirely with averages. In some cases the variations from these averages were rather wide, but in general the individual measurements were fairly close to the averages. Because of this fact and in view of the consistent relations shown by data from different localities and sets of cankers, despite the frequently small bases, a statistical presentation of means and deviations is not considered necessary.

AREAS OF PRODUCTION OF FRUITING STAGES

In connection with the growth measurements, observations were made on the extent and extension of the areas of the canker surface bearing aecia and pycnia. In general, the pycnial zone extends longitudinally to within one half to 3 inches and laterally to within one fourth to 1 inch of the outer limits of the discoloration, depending on the size of the canker and the time of year it is observed. The differences are naturally smaller on the smaller, slower growing cankers. As might be expected, the differences are generally greatest just before pycnia formation begins in summer, for from the beginning of canker growth in the spring the discoloration has been extending while pycnial activity has been quiescent. Thus, the differences are generally least at the height of pycnial activity.

Aecia are produced in the spring on the area which produced pycnia the preceding year. Part of this area may have produced pycnia for the first time 2 or 3 years earlier, and aecia for the first time 1 or 2 years earlier. The production of aecia from the same bark for 2 successive years is the usual occurrence in western white pine. On large stems it frequently occurs for 3 years in succession, and sometimes for 4 years or even more.

In this latter respect the rust considerably exceeds its performance described for *Pinus strobus* (*6, p. 515; 7, p. 29*), probably because of the thicker bark and greater food supply for the fungus in *P. monticola*. The aecial zone on *P. monticola* generally extends fairly close to the limits of the preceding year's pycnial zone and may coincide with it. Rhoads (*6, p. 515*) has recorded the production of aecia in the discolored area beyond the limits of the pycnial zone on *P. strobus*. The writer does not recollect having observed such an occurrence on *P. monticola*. Very frequently in this species, particularly when the canker is young and pycnia were produced for the first time the preceding year, aecia are not produced at all on the preceding year's pycnial zone, and sometimes the canker may have produced pycnia for 2 years or even more without producing aecia. Normally, however, when the aecia are not produced in the year following that in which the canker bears its first pycnia, they are produced the next spring (*5*).

INJURIOUS EFFECTS OF CANKERS

RELATION TO CANKER GROWTH

Most of the serious injury and killing result from mycelial activity on the trunk so far down from the tip that the death of a large section of the top or of the entire tree ensues. At such points the girdling growth is of chief importance. The higher on the trunk the infection is located, the greater importance downward growth assumes.

The rust infects its aecial hosts through the needles (*1*). In *Pinus monticola* needles are generally present only on the last few internodes of the trunk and branches. In its relation to injury, therefore, the infection of the trunk directly through the needles is relatively unimportant. Infection of the trunk occurs to some extent also through short shoots, the so-called "kurtztriebe" (*8, p. 33*), but although these are not uncommon in *P. monticola*, their insignificance in relation to the whole crown makes them comparatively unimportant as a source of infection. The effect of the fungus on its host depends to a large degree on the specific portion of the crown in which it grows. Serious injury and finally death are caused by eliminating from the life processes of the tree so large a part of the crown as to make further functioning impossible. The invasion of the trunk at a point at which the resulting girdling must end in the killing of a considerable part of the crown is accomplished through the centripetal growth of the mycelium downward from the point of infection in the branch (*7, p. 28*). In the branches, therefore, the downward growth of the canker is of first importance and lateral growth is relatively unimportant, as far as the life of the tree is concerned.

An exception to this general rule occurs in *Pinus monticola* when the infection is so heavy and the branch cankers are so numerous as to seriously damage or kill the tree through killing the branches. Such infection and killing are relatively uncommon, however, and are usually restricted to trees immediately adjacent to the infecting *Ribes* plants or close to *Ribes* concentrations, particularly when the species involved are of high susceptibility. Where large numbers of highly susceptible bushes occur in the stand, such killing of pines may become general in trees up to large pole size. Larger trees are less susceptible to killing in this manner, and in overmature veterans it has not been observed to occur at all. Veteran trees are found in many of the centers of oldest and heaviest infection in British Columbia. These trees have persisted through infection which has already wiped out the younger trees beneath them, and apparently the only manner in which such trees are killed or seriously injured is by spread of the fungus down the branches and into the bole.

The average rates of lateral and downward growths of cankers are compared in table 9, in which the Chee Kye and Thurston Bay canker measurements of 1924 and 1926 represent the optimum, and the Daisy Lake canker measurements of 1924 and 1926 represent slower growing conditions. These data are shown as curves in figure 6.

TABLE 9.—*Average annual downward and lateral canker growth rates as represented by measurements in 1924 and 1926 at typical areas in British Columbia*

Stem-diameter class (inches)	Chee Kye and Thurston Bay						Daisy Lake					
	Downward growth			Lateral growth			Downward growth			Lateral growth		
	Cankers	Average stem diameter	Canker growth	Cankers	Average stem diameter	Canker growth	Cankers	Average stem diameter	Canker growth	Cankers	Average stem diameter	Canker growth
	Number	*In.*	*In.*	*Number*	*In.*	*In.*	*Number*	*In.*	*In.*	*Number*	*In.*	*In.*
0.1–							38	0.13	1.05			
0.2–0.3	20	0.28	2.20				37	.22	1.30			
0.4–0.5	22	.44	2.47				1	.50	2.20			
0.6–1.0	10	.69	3.05				2	.70	2.35			
1.1–1.5	4	1.33	3.25	1	1.30	2.80	1	1.20	2.00			
1.6–2.0	8	1.76	3.79				2	1.75	2.70	1	1.60	2.00
2.1–2.5	2	2.50	3.20				2	2.30	2.45	2	2.30	2.15
2.6–3.0	4	2.88	4.05	2	2.85	2.75	6	2.83	2.72	5	2.84	2.52
3.1–4.0	12	3.56	4.46	9	3.60	3.21	13	3.66	2.91	14	3.64	2.59
4.1–5.0	6	4.60	4.70	5	4.68	3.44	6	4.77	3.57	6	4.77	2.55
5.1–6.0	3	5.33	4.20	4	5.48	3.48	3	5.53	3.43	3	5.53	2.70
6.1–7.0	4	6.45	4.80	4	6.60	3.80	1	6.10	3.60	1	6.10	2.40
7.1–8.0	1	7.90	4.60									
8.1–9.0										1	8.20	2.70
11.1–12.0	1	11.80	5.50	1	11.80	3.70						

Table 9 and figure 6 show that the averages of downward growth in the optimum areas range from a minimum of about 2 inches a year

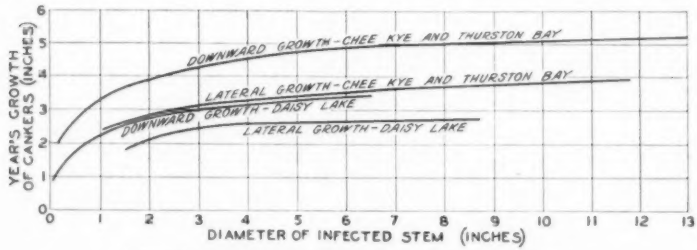

FIGURE 6.—Year's downward and lateral growth rates of cankers.

in the smallest diameter classes to a maximum of about 5 inches a year in the largest diameter classes. In the slower growing areas, the averages range from a minimum of about 1 inch per year to a maximum of about 3.5 inches per year.

Lateral-growth values are confined primarily to diameters ranging between 2.5 and 7 inches for the optimum areas and between 2 and 6 inches for the areas of slower growth. Within these diameter limits the growth averages range in the former areas from about 3 to between 3.5 and 4 inches a year, and in the latter from about 2.3 inches to between 2.5 and 3 inches a year.

Figure 6 shows that the curves for downward growth have the same general form as those for total longitudinal growth (figs. 2 and 4). In the smaller diameter classes the averages rise sharply to nearly 4 inches per year for diameters of 2 inches in the optimum areas and

to over 2 inches per year for diameters of 1 inch in the slower growing areas. Beyond these diameters they rise more slowly and level off to a more or less constant maximum of about 5 inches a year for diameters above 6 inches in the former areas and to a maximum of around 3.5 inches per year for diameters above 5 inches in the latter areas.

No data exist for lateral growth rates in the smaller diameters. The curves for lateral growth tend to assume, in the sections in which they may be compared with the curves for longitudinal growth, a form similar to the curves for downward growth, and it is probable that an extension of them on this basis into the smaller diameter classes would fairly accurately represent actual lateral growth values for these classes.

Since the distance to be covered peripherally is short in the smaller twigs and branches, these are girdled very rapidly. In twigs and branches having diameters approaching 1 inch, girdling generally occurs within a year after the canker makes its appearance. Beyond this diameter the time required for girdling is longer. In stems over 3 inches in diameter, the number of years required for girdling is in general roughly equal to the number of inches of diameter.

In the smallest twigs the portion of the twig beyond the canker is usually killed within 1 or 2 years following girdling, or within 2 or 3 years following the appearance of the canker in this class of stems. The death of the portion of the stem beyond the canker is termed "flag formation" because of the conspicuous red-brown color of the dead foliage of the affected part. In the intermediate branches and smaller trunks, this killing of the part beyond the canker, or flag formation, usually occurs within 2 or 3 years following girdling, but in some cases it may require as much as 4 years or more. In the larger trunks it generally occurs within 1 to 3 years. The greater weakening which occurs during the longer period required for girdling large trunks accounts for the greater rapidity with which death follows such girdling.

Death of the stem below the canker does not occur at the same time as the death of the portion beyond, except where the canker is located so far down as to kill the entire branch, or the entire tree in the case of infection on the trunk. Where the stem below the canker is not killed, downward growth of the fungus continues for an indefinite period depending on the number, thrift, and disposition of the feeding branches below, with further die-back following irregularly behind its downward advance. If the branches below are sufficiently numerous, vigorous, and closely disposed, the fungus will continue its downward growth until, in the case of infection on the branches, it reaches the bole; or until, in the case of infection of the trunk, it kills the tree.

Where the lower limits of the canker have progressed for a considerable distance down an internode, it is a common occurrence for the die-back to include the upper portion of the internode. The lower portion with the living remains of the canker at its upper end is thus left without any source of food supply from above. In these cases, despite this condition, the lower portion of the internode regularly remains alive for some time, permitting the fungus to continue its spread toward the region of the living branches in the next whorl below.

Investigations not yet reported, in which cankered branches were cut off with a knife near the lower limits of the canker, and uncankered branches were cut to form similar uninfected stubs, suggested that the presence of the infection at the end of the stub had the effect of stimulating a reversal of flow of the elaborated food materials. The cankered stubs remained alive, and downward growth of the infection on them continued for periods ranging up to several years, while the uncankered stubs almost invariably died within a few months.

In flag formation there frequently appears to be a partial decline of the stem for some distance below the canker, and the portion of the internode remaining below the die-back usually does not continue to live as long as did the cankered stubs. It generally remains alive for a full growing season, however, and commonly for as much as 2 years.

Despite the factors favoring its downward progress, the canker very often encounters an internode over which it is unable to pass, and the infection dies out. This is a particularly frequent occurrence in the smaller trunks or in the tops of the larger trees. The internodes of the trunks are normally longer than elsewhere in the tree, and where the diameter is small enough for girdling to occur within a few years, the fungus is unable to make sufficient downward progress, by the time the portion of the leader above the point of girdling dies, to traverse the remaining portion of the internode before the latter also succumbs. In portions of the trunks having larger diameters, where greater periods are required for girdling, internodes of similar length may be traversed easily.

In the smallest twigs and branches, internodes greater than 10 inches in length will generally halt the downward advance of the canker. In branches one half to 1 inch in diameter the infection generally dies out on internodes greater than 12 to 15 inches in length. On the larger branches still greater lengths of internode may be traversed; but since, where the stem is large, most of the foliage of the branch is beyond the canker, the entire branch is likely to die at the time of flag formation. Frequently the inner side branches have been partially or completely killed by shade, resulting in a length of partly or entirely unsupported stem over which the infection would be incapable of passing under any circumstances. This is particularly the case in the lower crown, where the inner branches have undergone the most intense shading and where all the branches are generally under suppression. On suppressed branches, even though the canker has not yet reached an internode that it cannot pass over, the whole branch may be killed at flag formation or within a short time afterward because of the combined influence of the canker and of suppression. Where the branch has few feeders or is otherwise in poor condition, it may likewise be killed within a short time; or it may be killed primarily from suppression or from some other cause aside from blister rust. When the branch or any part of it is killed from any cause, all the cankers on it or its affected portions are killed at the same time. Thus a large proportion of the individual infections are eliminated before they can reach the trunk.

Where the die-back is only partial and the mycelium is not completely killed, the rate of downward growth is variable. When the portion of the branch below the infection remains vigorous, the growth is entirely comparable to that on stems of similar size and condition in which no die-back has occurred. As already stated, at the time

of flag formation and for some time previous there is a tendency toward decline in the portions of the branch below the canker as well as above it. Measurements of cankers on the branches which remained entirely alive for the year following marking at Revelstoke in April 1928 (p. 482) showed that this loss of vigor may occur to such an extent as to retard the growth of the fungus. These measurements included only a portion of the cankers originally marked. In the remainder, complete longitudinal measurements were prevented either by flag formation or death of the entire branch. Where flag formation occurred, however, measurements were continued of the downward growth of the cankers. These measurements are the only values secured on downward growth following flag formation. They are arranged by stem-diameter classes in table 10. Similarly arranged measurements of cankers on branches which remained entirely alive are included for comparison.

TABLE 10.—*Average downward growth from Apr. 22, 1928, to Oct. 8, 1929, of cankers on branches partly destroyed by flag formation in 1928 and 1929, and on branches that remained entirely alive during the same period, at Revelstoke, British Columbia*

Stem-diameter class (inches)	Condition of affected branch: Portion beyond canker died during 1928 growing season					Portion beyond canker died during 1929 growing season					Entire branch remained alive				
	Cankers	Average stem diameter	Canker growth: Apr. 22 to Sept. 23, 1928	Canker growth: Sept. 23, 1928, to Oct. 8, 1929	Canker growth: 1928 growth as percentage of 1929 growth	Cankers	Average stem diameter	Canker growth: Apr. 22 to Sept. 23, 1928	Canker growth: Sept. 23, 1928, to Oct. 9, 1929	Canker growth: 1928 growth as percentage of 1929 growth	Cankers	Average stem diameter	Canker growth: Apr. 22 to Sept. 23, 1928	Canker growth: Sept. 23, 1928, to Oct. 8, 1929	Canker growth: 1928 growth as percentage of 1929 growth
	No.	*In.*	*In.*	*In.*	*Pct.*	*No.*	*In.*	*In.*	*In.*	*Pct.*	*No.*	*In.*	*In.*	*In.*	*Pct.*
0.2-0.3	4	0.20	1.23	1.23	100.0	17	0.26	1.38	1.49	92.6	7	0.29	1.34	1.66	80.7
0.4-0.5	1	.40	2.20	2.40	91.7	5	.46	1.82	1.70	107.1	13	.44	1.78	1.77	100.9
0.5-1.0						4	.65	1.98	2.40	82.5	4	.73	1.93	2.48	77.8

Table 10 shows that downward growth has continued at rates fairly comparable to those maintained before flag formation. In the 0.2- to 0.3-inch diameter class, 1929 growth, as compared with 1928 growth, is relatively slower for cankers in which flag formation occurred in 1928 than for cankers in which flag formation occurred in 1929 or in the cases in which the entire branch remained alive. In the 0.4- to 0.5-inch diameter class this value is higher, but only a single canker is represented. The cankers in which flag formation occurred in 1929 show a proportionally slower growth in 1929 compared with 1928 than the cankers on branches that remained alive. As has already been shown, the 1929 growth of these latter cankers was relatively slower than their growth in 1928 because of the exhausted condition of the branches on which they occurred. Evidently the branches having cankers in which flag formation occurred were generally even more exhausted.

In the cankers in which flag formation occurred in 1929, where there is a fair basis in number of cankers, a very wide variation in

1929 growth as compared with 1928 was evident. In some cases it was far below 1928 growth, in others far above. This is shown in table 11, which gives individually the comparative 1928 and 1929 growths for these cankers and for those on branches that remained entirely alive.

TABLE 11.—*Downward growth of individual cankers in 1928 and 1929 on branches partly destroyed by flag formation in 1929 and on branches that remained entirely alive during 1929, at Revelstoke, British Columbia*

Branch beyond canker died during growing season of 1929			Branch remained entirely alive in 1929		
Stem diameter (inches)	Canker growth		Stem diameter (inches)	Canker growth	
	1928	1929		1928	1929
	Inches	*Inches*		*Inches*	*Inches*
	0.7	1.0	0.2	1.3	1.6
	1.5	1.0		1.6	2.8
0.2	1.5	1.4		1.5	1.5
	1.2	1.1	.3	1.1	1.2
	1.2	1.5		1.6	1.3
	1.6	.4		.9	1.3
	1.6	1.8		1.4	1.9
	1.8	1.2		1.8	2.0
	1.3	.9		1.7	1.6
	1.3	4.3		1.7	1.6
	1.9	.8		1.7	1.6
.3	1.2	1.8	.4	1.7	1.5
	1.3	1.8		1.5	1.6
	1.7	.7		2.0	1.3
	1.7	2.1		2.2	1.9
	1.1	1.6		1.8	1.5
	.8	1.9		2.1	2.4
.4	2.1	1.2	.5	1.4	2.0
	1.9	1.7		1.2	2.0
	1.9	2.2		2.4	2.0
.5	1.6	1.5		1.7	2.5
	1.6	1.9	.7	2.1	2.7
.6	3.0	1.6		1.7	2.6
	1.5	2.5	.8	2.2	2.1
.7	2.2	3.0			
	1.2	2.5			

Table 11 shows that there was a far greater variation among the cankers in which flag formation occurred than among those on branches that remained alive. Evidently in some of the former the occurrence of flag formation was followed by a stimulation or release from retardation of downward growth. Apparently the sickly condition of the portion of the branch beyond the canker constitutes a decided drain on the healthy portion below the canker. This drain is eliminated when die-back occurs and the outward flow of water into the dying portion is stopped. The remaining portion of the branch recuperates sufficiently to permit downward growth of the fungus again to become normal. This tendency toward recovery following flag formation has been a common observation where the canker was not too far down on the branch and where the condition of the branch was unaffected otherwise than by the canker.

Where the canker occurs too far down on the branch or the branch is under the influence of suppression, a continued decline instead of recovery is the rule. The effect of this decline is apparent in table 11 where the 1929 growth-rate value is below that of 1928.

Although no measurements are available as proof, the writer has observed under certain conditions what appeared to be a decided

tendency of downward growth to accelerate with the approach of death in the affected tissues. This tendency was apparent where an entire branch, otherwise unaffected, was being killed by the canker and also where the canker following flag formation had not quite traversed a long internode which was about to succumb and below which the branch was vigorous. This apparent acceleration seems to suggest some inherited physiological reaction of the parasite which would bring the mycelium in contact with a better source of food supply and thus perpetuate the life of the organism in the host.

METHOD OF CALCULATING TIME AND MANNER OF INJURY

From the known rates and habits of canker growth, it is possible to calculate and estimate the probable development of the individual infection. This permits, far in advance of the occurrence, calculation and fairly accurate prediction of the time and extent of injury that will result from any given condition of infection on the pines. Special studies, not yet reported, have been made in this connection. This application of the knowledge of canker growth rates and behavior, however, may be briefly illustrated by a hypothetical case, as follows:

A young canker has entered from a side twig on a lower branch of good condition on a vigorous young tree 30 feet high in a moderately dense stand in the optimum pine range. The canker is 3 feet from the trunk. The diameter of the branch below the canker is 0.7 inch, and at its juncture with the bole is 1.0 inch. There are numerous "feeders", or living side branches, below the canker. The internodes are short enough for the canker to grow over. The diameter of the bole at the point of juncture with the branch is 4.0 inches. By referring to the curve of downward growth for optimum areas (fig. 6), it is found that the rate of downward growth on the branch should average about 3 inches a year. At this rate is would take 12 years for the infection to reach the bole. It is necessary next to visualize the probable changes that will take place in the tree during this time. At the present rate of growth it is calculated that the stand will have increased in this time by an average of about 20 feet in height and it is probable that within 5 years the stand will be closed and that this branch will have become suppressed. It is practically certain that the effects of suppression and the canker together will kill the branch and that the canker will consequently die before it can reach the bole.

Branch cankers high up in the upper crown of the same tree might be thought to be relatively innocuous, since so little of the crown is involved above them; but this is not the case. For example, a canker may occur 2 feet out on a branch at 22 feet from the ground, which has sufficient feeders, etc., to permit the canker to grow down to the trunk. The diameter of the branch below the canker is perhaps 0.3 inch, and at its juncture with the bole 0.6 inch. The diameter of the trunk at this point is 1.6 inches. The average annual growth of the trunk is 20 inches in height and, at the point at which the infected branch enters, 0.4 inch in diameter. It is calculated that the downward growth of the canker will average about 2.4 inches per year and that it will require about 10 years for the canker to reach the trunk.

By this time, if the growth in height maintains its present pace—and there is every indication from the growth of older trees that it will—the tree will be about 47 feet high. Its diameter at 22 feet will have increased to about 5.5 inches. It will then require about 5 years for the infection to girdle the tree; during this time growth in height may be retarded by the infection, but will amount to about 7 feet more. During these years the base of the crown has moved up from near ground level to about 15 feet above ground level, owing to suppression of the lowest branches. Thus, at the time of girdling the tree will be about 55 feet high, and the girdling, at 22 feet, will be near the base of the crown. Obviously the tree will be killed.

For comparison with this last case, let it be assumed that on the same branch the infection is located within 10 inches of the bole instead of 2 feet out. The diameter of the branch below the canker is 0.6 inch. The average downward growth rate of the canker is calculated as 2.7 inches per year. In 4 years it will have reached the bole. The diameter of the latter at the point of juncture with the branch will then be about 3.5 inches. It will require about 3 years for tne infection to girdle it. In the 7 years required for growth down the branch and girdling, assuming that growth in height was retarded by the girdling action of the fungus to an average of about 15 inches per year during the last 3 years, the height of the tree will have increased about 11 feet, to a total of about 41 feet. In this case, allowing 2 years for the death of the leader following girdling and 1 more foot of growth in height during this time, about 20 feet of the leader will be lost after the tree has attained a height of about 42 feet, and the tree will be killed or at least so seriously damaged as to be worthless.

Assuming that the infection occurred directly on the stem, about 12 feet of the leader would be killed after the tree had attained about 34 feet in height. This also would be serious, but the tree would probably recover; for it is unlikely that the canker would be able to grow down over the 20-inch internode below it and the infection would die out with the die-back of the leader. In this case a volunteer would take the place of the old leader, and the tree, if free from further infection, might still make a good timber tree.

If the annual growth of the tree were slow in the last two cases, averaging, for example, only 8 instead of 20 inches in height, and 0.2 instead of 0.4 inch in diameter, the sections of the leader killed would be only about 13 feet and 9 feet after the tree had reached the height of about 35 feet and 31 feet, respectively. In these cases, however, the fungus after reaching the bole would easily be able to span the 8-inch internodes below, and the infection would undoubtedly continue its downward spread on the trunk until the tree was destroyed.

It may therefore be concluded that infection at practically any point on the bole will usually kill or render worthless trees of the height class used in the examples or smaller trees.

In large trees approaching or past maturity, infections near the tip are relatively harmless. In mature trees growth in height is generally slower than in trees of the younger classes. Consequently the length of the leader above the canker increases less prior to girdling, and smaller sections of the leader are killed from cankers originating at similar positions in the tops of such trees than in the tops of younger trees. Furthermore, the interference with growth in height is less important in trees of the older classes, because they have already

developed to merchantable size; whereas, in the younger classes, continued growth in height is essential to the production of timber. Owing to the much greater length and size of crown in the larger trees, correspondingly greater lengths of the leader may be lost without killing or greatly retarding the growth of such trees. Therefore, since most of the timber volume is in the lower bole, considerable lengths of the leader may be killed in large trees without serious loss in merchantable volume or great reduction in volume increment, whereas in the small trees the infection prevents the production of timber.

In order to kill or cause serious damage in a large tree, infection must generally gain entrance to the bole at a considerable distance below the top. Since the lower branches of the crown are longer and their needles through which infection occurs are consequently farther from the bole, cankers in these branches are normally farther from the bole than are the cankers in the upper branches or in smaller trees. Because of the greater distance to be traversed and the greater chance that the canker will encounter an internode over which it will be unable to pass, and because of the relatively smaller number of feeding side branches close to the bole and the greater chance that the canker will kill the entire branch and itself at the same time, a much smaller proportion of cankers gain entrance to the bole from the older branches than from the younger branches.

Furthermore, the cankers that gain entrance to the bole in the vulnerable portions of the larger trees usually have a greater distance to travel than do the cankers in the younger trees, and the diameter of the bole is of course much greater. Therefore, a greater number of years is generally required for the infection to reach and girdle the bole and to kill or seriously damage the larger trees.

This does not mean that damage will not readily occur in mature trees of large size. Studies indicate that an average of 10 to 20 cankers is required to kill or seriously injure these larger size classes, whereas 1 canker suffices to kill the smallest tree. On the other hand, the much larger crowns of the large trees present a far greater target for the sporidia than do the crowns of the small trees. In spite of the fact that the crowns of the large trees are higher from the ground and consequently more removed and less directly exposed to the sporidia from the infected *Ribes* plants below than are the crowns of the smaller trees, there is a very rapid increase in the total number of cankers with increase in the size of the tree. In general this increase in the total number of cankers more than offsets the difference between the large and small trees in the number of cankers required to kill or seriously injure. The former, therefore, are as much predisposed to killing and serious injury as are the small trees. The injury merely requires a longer period to become effective.

The injury to trees of intermediate size is intermediate between that of the larger and that of the smaller trees. The foregoing examples, however, will suffice to illustrate the general application of the data on the development of cankers in relation to their injurious effects. From these data the amount of injury to any particular tree may be calculated.

Besides the data already given, information was secured on certain phases of canker development which, though of secondary importance with relation to the mode of injury, are of interest in connection with

the general description of the cankers. These phases concerned the rate and extent of swelling or constriction.

SWELLING AND CONSTRICTION

The rate of swelling of the cankers, as measured by the increase in the diameter of the stem at the center of the canker, ranged from 0 to 0.3 inch per year, depending on the age of the canker and the size of the part infected. Swelling commenced with the incipient stages of canker formation but did not usually become pronounced until after the discoloration had girdled the stem. As previously mentioned girdling usually occurs within a few months on the smaller branches, whereas on the larger stems it requires a period of years. As the rate of swelling in proportion to the rate of longitudinal growth is most rapid in the younger, smaller stems, the amount of swelling in proportion to the length is greatest in young cankers on such stems. After girdling on the smaller twigs and branches, the canker rapidly develops a short fusiform shape, which is maintained for a time; then the rate of swelling slows down, and as longitudinal growth continues the canker becomes greatly elongated. The greater the size of the stem, the slower is the rate of swelling and the less fusiform is the shape of the canker. On the larger stems the proportion of swelling to length of the canker is slight or negligible and diameter growth of the wood may ultimately be so reduced that the canker assumes the form of a constriction.

Except on the smallest twigs and branches, the bark of the canker generally becomes roughened and cracked in old age. The early death of the part infected usually prevents development to this stage on twigs and small branches. In the larger stems, particularly on the trunks, this cracking of the bark is frequently accompanied by resin flow, which in the case of old cankers on the larger trunks is often heavy.

Constriction, as described by Rhoads (*6, pp. 519–520*), begins with the reduction of the wood increment beneath the infected bark. He found that either retention of normal outline or constriction was the rule in *Pinus strobus* for cankers on stems 2 inches or more in diameter. As a rule, the infected stems retained their normal diameter for a time and became constricted only after the cankers had developed for a long time. Stem analyses showed that although the bark swelled in the earlier stages of the development of the canker, the reduction of wood increment beneath resulted in restoring the normal outline of the twig or in producing a constriction.

In *Pinus monticola* this process is not ordinarily found at such small diameters and the development of noticeable swelling is the rule at stem diameters up to 4 or 5 inches and is frequently found at even larger diameters. In the small twigs and branches there is frequently in the beginning a swelling of the outer xylem elements as well as of the bark, so that after the branch has died and the bark has sloughed off, the portion of the canker developed during the earlier stages of the infection is often still clearly evident as a fusiform swelling of the decorticated stem. Such swelling was apparently not noted by Rhoads in *Pinus strobus*.

During a trip through infection areas of the eastern part of the United States in 1927 the writer was struck by the frequent occurrence of exaggerated constrictions in *Pinus strobus* caused by the rust

on trunks ranging mainly from about 2 to 6 or 7 inches in diameter. These trunks had been girdled for some years, and diameter growth at the canker had been completely stopped. The bark tissues appeared to be almost or entirely dead and were largely impregnated with resin. The outer layers of the sapwood also were dead or dying and becoming impregnated with resin, but the inner portions were still alive and functioning. While downward conduction had been almost or entirely blocked, upward conduction had been maintained to such an extent that the leader above had continued to grow almost normally in height and diameter, except for a short distance just above the canker, where the stoppage of downward conduction had resulted in an acceleration of growth in diameter. Below the canker, the growth in diameter had also continued, but generally more slowly. The elaborated food materials for this latter growth had apparently been provided primarily from portions of the crown below the canker. In some cases where most of the infected bark had died, the growth, particularly above the canker, evidenced a distinct attempt at callus formation to heal over the affected portion of the stem. Some of the most exaggerated of these constrictions showed diameters above and below the canker of over twice that of the affected portion. Naturally these constrictions formed points extremely susceptible to breakage, and many trees had been broken at such points by wind and snow.

In *Pinus monticola* formation of such constrictions and resultant breakage has been as yet relatively rare, although breakage is common at the point of the canker in the larger tops and branches after the portions beyond the canker have been killed and rot fungi have found an entrance.

The reason for these differences in canker formation and development in the two species probably lies in the fact that the living bark tissues are generally thicker in *Pinus monticola* than in *P. strobus* and thus provide a food supply that is less readily exhausted by the fungus and consequently a substratum that is less readily killed.

SUMMARY

The growth of over 400 cankers of *Cronartium ribicola* Fischer on *Pinus monticola* Doug. was measured in six different areas in southwestern British Columbia, representing a variety of climatic and environmental conditions for the native range of the host. The cankered stems ranged in diameter from the smallest twigs to trunks up to 8 inches, and in conditions from poor to thrifty.

Size of the infected stem (i.e., twig, branch, or trunk) and regional site conditions were the predominant influences in canker growth rates. Vigor of the infected stem was of relatively little importance.

The curves for longitudinal growth were parabolic in form, rising steeply between stem diameters of 1 to 2 inches, and tending to become almost horizontal at diameters beyond 5 inches.

An average of about 85 percent of the year's growth took place in the cankers from spring to fall.

The cankers grew more rapidly downward than upward, apparently because the diameter of the lower portion of the stem is normally greater than that of the upper portion. On the trunk, which tapers slowly, downward growth of the cankers averaged nearly the same as upward growth; on the smaller, slower growing twigs, which taper

rapidly, downward growth was over 30 percent greater than upward growth. In optimum areas the average annual downward growth ranged from about 2 inches on the smallest twigs to about 5 inches on stems more than 6 inches in diameter; in similar stem-diameter classes, in the areas where growth was slower, average downward growth ranged from about 1 inch to between 3½ and 4 inches per year.

Lateral or girdling growth was measured only on trees of the larger stem classes. In trees of the largest stem diameters the average rates of lateral growth ranged to about 3½ inches a year in the optimum areas and to between 2½ and 3 inches in areas of slower growth.

In the smallest stems, girdling occurred within a few months during the growing season. In the larger stems the average number of years required for girdling was about the same as the number of inches of stem diameter.

Aeciospore production continued regularly for several years in succession on the same areas of bark.

The stem is generally killed down to the lower portions of the canker within 1 to 4 years following girdling. If the canker occurs far enough down on the stem, or if the stem is in a weakened condition, the entire branch or trunk may succumb at the time of this first killing or shortly afterward. Otherwise the canker continues its downward growth with further die-back following irregularly behind it. Frequently this progressive die-back takes in the canker itself. In this manner, and also through the death of entire branches owing mainly to the action of the canker and of suppression, great numbers of the cankers die out before they can reach the trunk. The dying out of cankers is greatest on the larger trees where the branches are longer and the lower and inner portions of the crowns are under suppression. On the other hand such trees with their large crowns are more exposed to infection, which in general results in a greater number of cankers.

Most of the serious injury and killing of trees results from girdling well down on the trunk by cankers that have grown down from the branches. Examples are given of the application of the data on canker development to calculations of the time element and the manner of killing or injury under determinable conditions.

Data are presented on canker swelling. The most pronounced swelling occurs on the smallest stems and on these only during the early stages of canker development. In stems over 5 inches the occurrence of swelling is negligible, or constriction develops.

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RUN-OFF AND EROSION FROM PLOTS OF DIFFERENT LENGTHS[1]

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INTRODUCTION

It has been a common observation among farmers that erosion seems to be more serious on long slopes than on short ones. Probably it has also been assumed that the amount of run-off is greater on long slopes, although less attention has been given to this phase of the subject. As a matter of fact, little definite information is available on these points.

Probably the first tests to determine the effect of length of slope on run-off and erosion were made by Bartel.[2] Work is now in progress at a number of Federal erosion stations, and the preliminary results have been included in the annual reports of these stations. A brief summary of the results has been reported by Middleton and Byers,[3] and Bennett.[4] A study of these results reveals no very consistent trend in the rate of erosion or run-off from slopes of different lengths. Because of this apparent lack of consistency and the consequent need for more definite knowledge, the present work was started. By using methods whereby all factors could be more carefully controlled it was thought that more definite results might be obtained, even though the plots used were smaller than those employed in other tests.

PLAN OF THE EXPERIMENTS

Two sets of experiments were made. In each, 4 plots 3 feet wide and 10, 20, 40, and 100 feet long were used (fig. 1). These were surrounded by strips of galvanized iron set in the ground to a depth of 6 inches. There was a 3-foot alley between plots, and the middle of all plots was on a line at right angles to the direction of the length of the plots. By this arrangement the soil conditions in each plot should be representative of those prevailing over the whole of the small area employed in the experiments. The slope of the land was 4 percent in experiment 1 and 4.4 percent in experiment 2. The plots used in the first experiment were only about 25 feet south of those used in the second.

The soil in which the tests were carried on is Derby silty clay loam. This soil has a fairly permeable surface soil and the subsoil is a silty clay to clay in the B horizon, but has not developed anything that even approaches a clay pan. The soil and subsoil could, therefore, be considered as having characteristics that would permit rather rapid absorption. The surface of the soil was kept free from vegeta-

[1] Received for publication Oct. 17, 1933; issued June, 1934. Contribution No. 228, Department of Agronomy.

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tion and was hoed and raked before the beginning of each series of tests. When several runs were made on the same or succeeding days the soil could not be recultivated and was sometimes considerably packed by the applications of water.

The water was applied artificially by means of sprinkling cans to simulate rainfall in the same way that it was applied by Duley and Hays.[5] In three cases the run-off from natural rainfall was collected. In order to get an even distribution of water over the plots several men sprinkled the water simultaneously. With the heavier applications eight men were employed on the 100-foot plot, each man being assigned a 12.5-foot space over which he distributed an allotted quantity of water in a given time. On the shorter plots each man was given 10-foot spaces. By properly calibrating the sprinklers it was

FIGURE 1.—Plots used in experiment 1. A portion of the 100-foot plot is shown at the left, and the 40-, 20-, and 10-foot plots at the right. Three applications of water were made at the rate of 1 inch in 30 minutes.

possible to put on the water in almost exactly the time desired on all plots.

The losses were determined by weighing the total amount of run-off and then sampling. The samples were oven-dried and the amount of soil and water calculated.

RESULTS

RUN-OFF

A summary of the results obtained will be found in table 1 and figures 2 and 3. These results show that the percentage of run-off was in general greater on the short plots, and decidedly greater when the average of all plots is considered. On two occasions, July 12 and 27 in experiment 2, the 20-foot plot absorbed water much more rapidly than the others. No satisfactory explanation for this has been found, but the greater absorption was very noticeable while the water was being applied. It is felt that these two results should not be included in the data since they were obviously inconsistent at the time. However, averages have been given both with and without them.

[5] DULEY, F. L., and HAYS, O. E. THE EFFECT OF THE DEGREE OF SLOPE ON RUN-OFF AND SOIL EROSION Jour. Agr. Research 45: 349–360, illus. 1932.

TABLE 1.—*Effect of length of slope on run-off and soil erosion*

EXPERIMENT 1

Date	Water applications or rainfall	Run-off from plots indicated				Soil eroded per acre from plots indicated			
		10-foot	20-foot	40-foot	100-foot	10-foot	20-foot	40-foot	100-foot
1931		*Percent*	*Percent*	*Percent*	*Percent*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*
Dec. 23	1 inch in 30 minutes	0.00	1.26	0.87	0.09	------	121	21	1
Do	1 inch in 60 minutes	42.69	67.11	55.39	43.47	3,071	5,242	1,757	704
Dec. 26	½ inch in 30 minutes	7.94	6.43	8.56	5.43	160	109	174	86
Do	1 inch in 60 minutes	64.96	74.37	65.72	59.92	378	1,271	1,078	794
1932									
Mar. 28	1 inch in 60 minutes	29.80	20.57	15.12	15.36	270	274	134	171
Do	1 inch in 30 minutes	77.03	60.11	58.52	57.04	1,253	2,029	1,073	2,607
Apr. 26	0.32 inches rain	25.20	42.70	21.10	16.20	159	558	289	253
May 11	1.26 inches rain	27.60	26.70	19.30	18.30	4,980	3,005	1,230	1,350
Dec. 3	1 inch in 30 minutes	40.54	34.90	28.63	25.24	1,086	423	479	299
Do	1 inch in 60 minutes	26.81	24.59	16.75	7.02	244	203	171	60
1933									
Apr. 24	1 inch in 30 minutes	1.59	1.43	.81	1.20	18	18	12	30
Do	----do	40.38	29.14	26.78	28.15	735	415	491	912
June 6	1 inch in 15 minutes	44.60	31.02	25.56	30.30	271	847	1,085	3,107
June 8	----do [a]	37.51	29.73	29.41	25.00	697	900	1,082	2,614
	Average or total	33.33	32.15	26.61	23.77	13,322	15,415	9,076	12,988
	Average for applications of June 6 and 8.	41.05	30.38	27.49	27.65	------	------	------	------
	Total for applications of June 6 and 8.	------	------	------	------	968	1,747	2,167	5,721
	Total for all applications except for those of June 6 and 8.	------	------	------	------	12,354	13,668	6,909	7,267

EXPERIMENT 2

1933									
July 8	1.5 inches in 35 [b] minutes (rainfall)	46.42	37.69	30.61	29.22	1,364	3,092	4,403	4,829
July 12 (a.m.)	1 inch in 60 minutes	21.33	5.98	14.49	7.74	174	102	199	137
July 12 (a.m.)	1 inch in 30 minutes	55.62	38.01	49.39	36.66	769	718	943	1,000
July 12 (p.m.)	----do	54.58	34.65	40.45	33.29	943	573	903	1,197
July 19 (a.m.)	1 inch in 15 minutes	52.62	40.00	38.38	33.98	4,646	3,920	4,827	4,907
July 19 [c]	----do	65.64	57.41	56.79	56.03	5,517	5,009	6,316	5,866
July 27	1 inch in 30 minutes	10.65	7.33	9.30	5.48	116	232	421	160
	Average or total	43.84	31.58	34.20	28.91	13,529	13,646	18,012	18,096
	Average or total omitting results for July 12 and 27 [d].	54.89	45.03	41.93	39.74	11,527	12,021	15,546	15,602

[a] This application followed an application of 1 inch in 30 minutes which gave no run-off.
[b] Only part of 3.25 inches rain collected.
[c] 6 minutes after first application July 19.
[d] Omitting results for July 12 and 27 on all plots. The 20-foot plot took in water differently from the others on these days and no satisfactory explanation has been found.

When the results are analyzed in detail it will be seen that the run-off from the shorter plots exceeded that from the longer ones in 69 cases out of 84 in experiment 1. If calculations are made according to the method of Salmon,[6] it will be found that D/E = 7.5, which indicates that the difference is probably not due to chance. In experiment 2 the run-off from the shorter plots exceeded that from the longer ones 27 times out of 30, or D/E = 6.6, which again indicates that the difference is not due to chance. If the results from both tests are combined, the run-off from the shorter plots is greater in 96 cases out of 114, or D/E = 10.9, from which it is safe to conclude that a larger percentage

[6] SALMON, S. C. THE POINT BINOMINAL FORMULA FOR EVALUATING AGRONOMIC EXPERIMENTS. Jour. Amer. Soc. Agron. 22: 77-81. 1930.

run-off may be expected from short slopes than from longer ones, at least up to the lengths used in these tests. This was true during light applications of water such as those of June 6 and 8 and July 19, 1933.

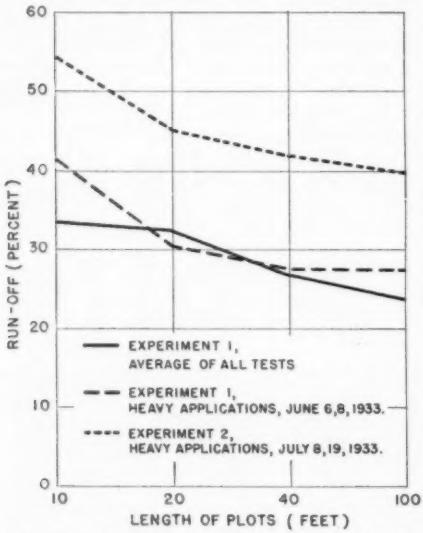

FIGURE 2.—Percentage run-off from soil plots of different lengths.

The explanation for the greater absorption on the longer plots probably lies in the fact that when water is running over a long slope there is more time for it to be absorbed than on a shorter slope. Unless a soil had reached its absolute water-holding capacity this factor would continue to operate. The results of these tests, like those of Duley and Hays,[7] indicate that field soils on sloping ground are seldom if ever so thoroughly saturated that they fail to absorb some water.

The decrease in run-off was less between the 40-foot and 100-foot plots than between 40-foot plots and the 10- or 20-foot plots. This was probably due to the fact that during heavy applications of water much of the lower part of the long plot was covered with a sheet of water. The percentage of run-off was thereby increased since absorption was already going on at approximately the maximum rate. The importance of this factor probably would be greater with still longer plots, and might possibly reach a point where the percentage of run-off would increase with further increase in length of slope, particulary during very heavy rains, for if the entire surface were covered with water, any additional rainfall would give approximately 100 percent run-off.

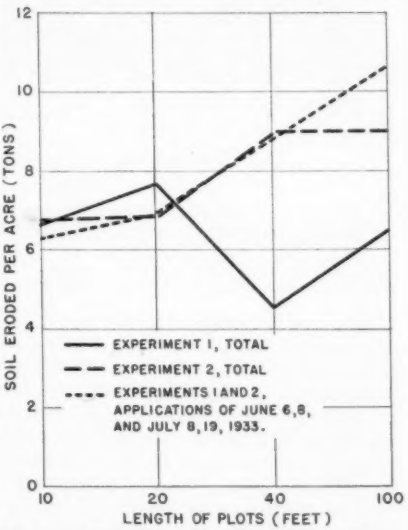

FIGURE 3.—Tons of soil eroded from soil plots of different lengths.

EROSION

The results of the erosion measurements for these plots were less consistent than the results for run-off. If the average of all results in experiment 1 are

[7] DULEY, F. L., and HAYS, O. E. See footnote 5.

considered for the different plots it will be seen that the figures for the 10-, 20-, and 100-foot plots are not widely different, but those for the 40-foot plots are low. The large amount of erosion on the 10- and 20-foot plots is due mainly to the results of the test of December 23, 1931, and the rain of May 11, 1932. There seemed to be a general tendency, when the amount of erosion was small, for the rate of erosion per acre to be relatively high for the short plots. This probably can be accounted for by the larger percentage of run-off. When the amount of erosion was large there was a tendency for it to be greater on the longer plots. This probably can be explained by the increased carrying power of water down the longer slope, as the water tends to concentrate in larger volume and to follow definite channels. This increase in volume with its greater cutting power is sufficient to overcome the effect of a larger percentage run-off on the shorter plots. If consideration is given to the times when the applications of water was most rapid, as on June 6 and 8 and July 19, 1933, when water was applied at the rate of 1 inch in 15 minutes, and the rain of July 8, 1933, when 1.5 inches fell in 35 minutes, it will be noted that erosion was heaviest on the longer plots.

If the results of all tests are considered, erosion from the longer plots is found to exceed that from the shorter ones in 61 out of 114 cases. Since $D/E = 1.1$, the results do not appear to be statistically significant. However, if the results for the very rapid rains are considered, erosion from the longer plots is found to exceed that from the shorter ones in 27 cases out of 30. $D/E = 6.6$, which indicates that the results are probably not due to chance. It must, however, be remembered that the numbers used in this case are small, and hence are of less value statistically. Nevertheless they do indicate a definite trend, and since it is the hard dashing rains that cause the greatest amount of erosion, these results have a more direct application to practical conditions.

In general the results here reported indicate that during the lighter applications of water the largest amount of erosion per unit area occurs on the short slopes, but with the heaviest applications the reverse is true. This finding may account for the fact that some of the results obtained in other experiments have shown more erosion from short plots, while in other experiments more was obtained from long plots. The relative amounts seem to depend upon the character of the rainfall.

SUMMARY

Soil erosion and surface run-off were measured on plots 10, 20, 40, and 100 feet in length, to determine the effect of slope length on these processes. Measurements were made on two such sets of plots.

The soil was a silty clay loam, free from vegetation and loose organic matter, and was surface-cultivated. The first set of plots had a slope of 4 percent and the second a slope of 4.4 percent. To simulate rainfall most of the water was applied with sprinkling cans; in a few cases natural rainfall was used.

There was a larger percentage of surface run-off from the short plots than from the long ones. This seemed to be true with both the heavy and light applications of water for the plot lengths under consideration.

The results for soil erosion were less consistent. When the rate of water application was light, there was a tendency for the erosion from the short plots to run relatively high as compared with the others. When the rate of application was heavy, i.e., 1 inch in 15 minutes, erosion was greater on the long plots. These results indicate that when rainfall is light short plots may possibly undergo the greater erosion, but when rains are heavy the reverse is true.

THE ABSORPTION AND EVAPORATION OF MOISTURE FROM PLANT CONTAINERS[1]

By LINUS H. JONES

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INTRODUCTION

The ordinary flowerpot made of clay and fired is familiar to everybody. Jones [2] has shown that plant containers of metal, glass, and paper will support plant growth equal to, and frequently better than that obtained in clay pots. The real difference between porous and nonporous pots is the property of the former to absorb and evaporate moisture. Soil aeration through the wall of the pot is not probable and is hardly possible.[3]

In seeking a scientific explanation for the inferiority of plants grown in clay flowerpots on a dry surface, the subject of water relations in the soil as influenced by the absorption of water by the pot and its evaporation from the pot seems to give a satisfactory answer. In nonporous plant containers, evaporation can take place only from the surface of the soil. In the porous pots, the pot itself acts as an evaporating surface; and as its area is considerably greater than that of the surface area of the soil, the effects of evaporation from such containers are accentuated. The plant container directly affects the water relationships in the soil mass. Jones, using high greenhouse temperatures, found that the loss of water from 3-inch porous flowerpots was twice as much as from nonporous containers of the same size, and that this increased loss of moisture by evaporation had a cooling effect on the soil mass.

PLAN OF THE INVESTIGATION

The investigation reported herein was planned to secure information that could be used as a scientific basis for the proper understanding of water movement in a clay flowerpot. The experiments were planned to determine the maximum absorbing capacity of clay flowerpots. Painted flowerpots and cement pots were included in the experiments concerned with evaporation. Though porous to air and moisture, the capillary structure of the cement pot is so coarse that unless the soil mass is quite wet, capillary continuity is broken between the cement pot and the soil and the pot will function as a nonporous one.[4]

ABSORPTION OF MOISTURE BY THE CLAY FLOWERPOT

When both the soil and the flowerpot approach dryness, any applied water is taken up by both. Apparently there are no figures to

[1] Received for publication Nov. 22, 1933; issued June, 1934. Published as Contribution no. 178 of the Massachusetts Agricultural Experiment Station.

[2] JONES, L. H. FLOWERPOT COMPOSITION AND ITS EFFECT ON PLANT GROWTH. Mass. Agr. Expt. Sta. Bull. 277, pp. [148]–161, illus. 1931.

[3] ———. AERATION OF SOIL IN PLANT CONTAINERS. Florist Exch. and Hort. Trade World 79 (11): 39, illus. 1932.

[4] JONES, L. H. CEMENT FLOWERPOTS . . . Florists' Exch. and Hort. Trade World 80 (2): 9, illus. 1932.

suggest the approximate amounts of water that should be applied daily to satisfy the needs of potted plants in flowerpots of various sizes. Table 1 shows the maximum amount of absorption that can take place in a clay flowerpot and the relation of the absorbed quantities to the amount of water usually applied to a potted plant.

TABLE 1.—*Normal weight of water applied to clay flowerpots of different sizes, the weight of water that clay pots can absorb, and the weight of water available for the soil and plant*

[Average for 20 pots]

Size of pot (inches)	Normal watering * applied	Water absorbed by pot		Water available for soil and plant	
	Grams	*Grams*	*Percent*	*Grams*	*Percent*
2	15	9.10	60	5.90	40
2½	35	14.10	40	20.90	60
3	50	20.55	41	29.45	59
4	100	38.85	39	61.15	61
5	200	83.15	42	116.85	58
6	300	130.30	43	169.70	57

* The amount of water that constitutes a normal watering was determined by averaging data from several sources. The amount of soil in the pot and the human element in watering will cause these figures to vary slightly. However, they represent a fair basis for investigational work.

A cement pot made from a mixture of sand and cement does not have so large a water-holding capacity as a flowerpot made of fired clay, consequently a larger percentage of the water applied to the soil in a cement pot is available for the plant. Table 2 shows a comparison of the absorbing capacities of clay and cement flowerpots.

TABLE 2.—*Normal weight of water applied to clay and cement flowerpots of different sizes, the weight of water absorbed, and the weight available for the soil and plant*

[Average for 2 pots]

Size of pot (inches)	Type of pot	Dry weight of pot	Normal watering applied	Water absorbed by pot		Water available for soil and plant	
		Grams	*Grams*	*Grams*	*Percent*	*Grams*	*Percent*
4	Clay	390	100	37	37	63	63
	Cement	406	100	23	23	77	77
5	Clay	643	200	83	41	117	59
	Cement	618	200	44	22	156	78
6	Clay	1,060	300	121	40	179	60
	Cement	1,032	300	79	26	221	74

In order to determine the amount of water that could be absorbed by a clay pot in the presence of a growing plant, the following experiments were performed: Twenty-four 4-inch pots were dried for 1 day at an air temperature of 33° C. and weighed. Each pot was planted with a geranium plant and placed in a greenhouse on a moist soil bench. At the end of 6 weeks the potted plants were transferred to a dry surface and the watering adjusted so that the soil was almost dry as the plants began to wilt. (This procedure is analogous to the conditions under which house plants are grown and watered.) When all 24 plants were reacting nearly the same and needed watering, they were equally divided into two series. The plants and soil were removed from the A series and the pots weighed to determine how

much water was retained by the pots. From table 3 it appears that the pots were as dry as when the experiment was started. To each pot of the B series 100 grams of water was applied and the series allowed to stand for 1½ hours. The pots with soil and plants were weighed and the loss of moisture by transpiration and evaporation determined. The soil and plants were then removed and the wiped pots weighed. Table 3 gives averages and calculations on which are based the conclusion that nearly one quarter of the water applied was absorbed by the pots. This figure represents about one half of the absorbing capacity of the pots (table 1). It was observed that in 1½ hours the water had penetrated the soil to a depth of but 1 inch and probably would not have penetrated more deeply as the capillary attraction of the pot for moisture was greater than that of the soil.

TABLE 3.—*Method of grouping the various weights of clay pots in order to obtain a general average of the percentage of water absorbed by them when containing plants*

Series and no.	Item considered	Weight
Series A (check):		*Grams*
1	Average initial weight of 12 pots	394.00
2	Average final weight of pots minus soil and wilted plants	393.00
Series B:		
1	Average initial weight of 12 pots	390.75
2	Average weight of pots plus soil when soil was dry and plants wilting	812.08
3	Average weight plus 100 grams of water	912.08
4	Average weight 1½ hours after water was applied	905.91
5	Loss in 1½ hours due to evaporation and transpiration	6.17
6	Average weight of empty pots 1½ hours after water was applied	413.33
7	Average weight of water in pots after 1½ hours (413.−33390.75)	[a] 22.58

[a] 22.58 percent of the total weight of water applied was absorbed by the clay pots in 1½ hours.

EVAPORATION OF MOISTURE FROM POROUS AND PAINTED FLOWERPOTS

Moisture loss from the soil in a porous pot takes place in two directions—the evaporation from the surface of the soil causes a vertical movement of soil moisture while the evaporation from the wall of the pot causes a lateral movement. If the porous pot is kept on a moist bench surface, some of this evaporated moisture is replaced from the moisture below the pot. The following experiment was conducted to determine the effect of pot environment (bench surface and air) on the quantity of water lost by evaporation from the surface of the soil and through the wall of the pot.

Flowerpots, 5-inch size, of clay, cement, and painted clay were placed on a dry bench surface of boards and on a moist bench surface of soft-coal cinders. Each type of pot for each condition was replicated six times. The soil, a compost, was sieved through a 1¼-inch mesh screen. The pots were filled level full and the soil firmed. The drainage hole in each pot was covered with a piece of broken pot, as is customary. The pots were allowed to remain on a moist surface for 20 hours for the natural adjustment of moisture through the soil before being weighed and were brought up to this initial weight every 24 hours, after first determining the weight lost. Table 4 shows the loss of weight for each one of the 14 days of the experimental period. Naturally, the amount of evaporation is affected by the weather conditions prevailing at the time. This influence is graphically represented in figure 1.

TABLE 4.—*Average loss of weight in 24-hour periods from clay, painted, and cement soil-filled pots on dry and moist surfaces*

Period (days)	Clay pots		Painted pots		Cement pots	
	Dry surface	Moist surface	Dry surface	Moist surface	Dry surface	Moist surface
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
1	53	18	13	11	27	5
2	63	29	17	16	30	21
3	64	28	15	11	27	18
4	66	36	19	18	31	25
5	69	41	18	18	33	23
6	53	26	15	11	27	18
7	54	18	16	11	30	[a] 2
8	56	23	18	14	29	14
9	28	5	10	8	19	[a] 10
10	53	17	17	12	27	5
11	44	15	14	11	24	7
12	48	14	14	12	22	5
13	11	[a] 6	4	4	5	[a] 10
14	20	5	7	5	11	[a] 1
Total	4,094	1,630	1,184	947	2,064	646

[a] Gain in weight.

The results indicate that the evaporation of moisture from porous clay pots on a dry surface was approximately three times greater

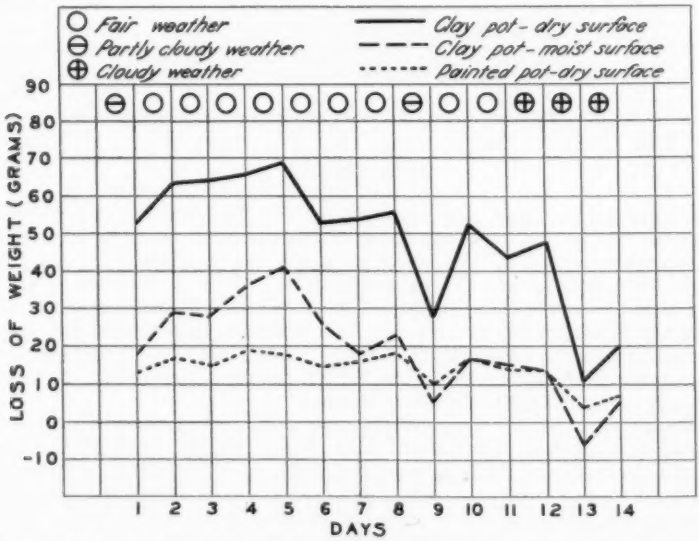

FIGURE 1.—Effect of weather on loss of weight of clay pots on dry and moist surfaces and painted pots on a dry surface. Average for six pots.

than that from painted pots. As the painted pots could evaporate moisture only from the soil surface, the data show that two thirds of the loss of moisture from the soil in a clay pot on a dry surface took place through the wall of the pot, indicating that there was twice as much movement of moisture laterally as vertically. When the porous clay pot was kept on a moist surface, a considerable propor-

tion of the water lost by evaporation was replaced from the moist surface. It is also possible in humid weather for the porous clay pot to absorb more moisture from the moist bench than is lost by evaporation.

The cement pot, because of its coarse structure, influences loss of moisture according to the ability of soil moisture to leave the soil and enter the pot wall. When moisture was first applied at the beginning of a 24-hour period, the pot was able to absorb it. As the period progressed, the moisture-retaining power of the soil became more effective than the pull of the pot. Thus capillary contact between soil and pot was broken. Hence, soil-moisture movement in a cement pot is more like that in a painted pot, vertical, than in a porous clay pot, vertical and lateral. Table 5 shows the loss of moisture in 1-hour periods from the three types of pots. The transition period, when the cement pot (on a dry surface) changed over from behaving like a porous pot, occurred at about the end of the fifth hour.

TABLE 5.—*Loss of weight in hourly periods from clay, cement, and painted soil-filled pots on dry and moist surfaces*

Period (hours)	Dry surface			Moist Surface		
	Clay	Cement	Painted	Clay	Cement	Painted
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
0–1	5	1	4	1	0	1
1–2	8	8	2	4	5	1
2–3	2	4	3	5	4	2
3–4	12	7	2	3	3	3
4–5	10	6	2	6	5	2
5–6	10	2	2	7	1	3
6–7	1	5	4	2	5	3
7–8	5	1	0	2	3	0
8–24	45	12	10	22	14	13
Total loss	98	46	29	52	40	28

DISCUSSION

The absorption of moisture by the clay pot and its subsequent evaporation into the air is a continuous process as long as there is any capillary moisture in the soil mass. Tests with the rubber vacuum disk proved that the word "porous" as applied to moist clay flowerpots should be limited to mean porosity to moisture. Air does not pass though the wall of a moist clay flowerpot. The process of lateral movement of moisture transports more water in this direction than is moved vertically and should, therefore, be accompanied by a corresponding lateral movement of soluble plant food. Associated with the movement of water in plant containers is the distribution of the root system of the plant. Where lateral movement is possible through a porous pot, the root system, for the most part, is found between the wall of the pot and the soil mass. On the other hand, if lateral movement is not possible, the root system ramifies through the soil mass with only a few roots developing adjacent to the pot wall.

Water relations, water movement, and root distribution are entirely dependent on the structure of the plant container. The culture of

potted plants should include practices that encourage a more or less even balance of water content in the environment of the roots. In a nonporous pot with a ramifying root system and lack of lateral movement of water, it is comparatively easy to maintain an even distribution of moisture in the soil mass. In a porous pot where the root system is almost entirely against the wall of the pot, it seems almost necessary to keep such pots on a moist surface. If a moist surface is not used, the pot itself will withdraw moisture from the nearest source which happens to be the very region where the roots have developed or are developing.

SUMMARY AND CONCLUSIONS

This investigation is concerned with the amounts of water usually applied to potted plants and the moisture-holding capacities of pots of various sizes. The loss of water by evaporation has been determined also for pots in certain specified environments.

Under conditions that frequently prevail with growing plants, nearly one quarter of the amount of water applied was absorbed by the flowerpots in 1½ hours.

Twice as much water was evaporated from the wall of a clay pot as from the surface of the soil. This would indicate that there was twice as much moisture moved laterally as was moved vertically.

When the clay pot was kept on a moist surface, a considerable proportion of the evaporated water was replaced from the supply of moisture beneath the pot.

Cement flowerpots did not have so large a water-holding capacity as those made of fired clay. The cement pot evaporated moisture from the wall as long as the wall could maintain a capillary connection with the soil mass. When such capillary contact was broken, the cement pot behaved as a nonporous painted pot.

The essential difference between a porous and a nonporous plant container is the ability of the former to evaporate moisture from its wall and replace this moisture from the soil moisture within the pot.

THE RELATION BETWEEN ABNORMAL ORIENTATION OF THE 4-DAY EMBRYO AND POSITION OF THE CHICK AT HATCHING [1]

By J. R. CAVERS, *research assistant in poultry husbandry*, and F. B. HUTT, *poultry husbandman and animal geneticist, Minnesota Agricultural Experiment Station*

INTRODUCTION

Despite the extensive knowledge at hand concerning the early embryology of the chick and the steadily accumulating information regarding its behavior at the time of hatching, there is very little known of the relationships which exist between the two stages of development. Because of their importance as factors causing embryonic mortality, malpositions of the fully formed embryo have been the object of study at this station for several years. The data herein presented deal with abnormal orientations of the young embryo and their possible effects upon the subsequent position of the chick within the egg.

MALPOSITIONS

The completion of the incubation period usually finds the body of the embryo parallel to the long axis of the egg, with the head towards the large end of the shell. It has been generally considered that the embryo accommodates itself thus to the shape of the egg at the end of the second week, but Kuo (*9*),[2] who has described the manner in which the final position is attained, found that the time of its fixation is usually about the tenth day.

Several well-defined deviations from the normal position at hatching, referred to as malpositions, have been described.

The method of designating normal and abnormal positions of fully formed embryos at this laboratory is as follows:

N. The normal position. Body parallel to long axis of egg and head in large end; beak under right wing and toward air cell (fig. 3, *A*).
I. Head buried between thighs.
II. Embryo upside down with head in small end of egg (fig. 3, *B*).
III. Head turned to left and away from air cell.
IV. Body rotated so that head is away from air cell; otherwise normal.
VI. Head not beneath wing, but above or away from it.

More complete descriptions of these malpositions, data on their frequencies, and discussions concerning their relation to embryonic mortality, have been given by Sanctuary (*12*), Hutt (*6*), and others. A detailed description of position VI has been given by Hutt and Pilkey (*7*). The malpositions differ in the extent to which they prevent or hinder hatching. Position I is undoubtedly always lethal, and positions III and IV appear to be fatal in nearly all cases, if not in all. Some of the embryos in positions II are able to hatch. Position VI is probably a considerable hindrance to the chick, if not an actual barrier to hatching.

[1] Received for publication Nov. 8, 1933; issued June, 1934. Paper No. 1224 of the Journal Series of the Minnesota Agricultural Experiment Station. The sixth paper of the series of "Studies in embryonic mortality in the fowl."

[2] Reference is made by number (italic) to Literature Cited, p. 530.

Although some chicks in malpositions may hatch and their positions then be unknown, the influence of abnormal orientations can still be studied in the embryos which fail to hatch.

In the first paper of this series, Hutt (*6*) suggested that some of the abnormal positions of fully formed embryos may result from the aberrant orientations of the embryo, which several embryologists have shown to be not infrequent, and which are known to be established at early cleavage. Such an origin seemed especially likely in the case of position II (head in small end of egg) since very young embryos are sometimes found parallel to the long axis of the egg (instead of at right angles to it), with the head pointing toward the small end. Accordingly an investigation was begun in 1931 to determine whether or not any relation exists between the early orientation and the later position of the embryo. Since the investigation was begun Taylor (*13*) has reported that from embryos originally abnormally oriented there resulted 50 percent more embryos in position II than there were from embryos having normal orientation at 6 days.

ORIENTATION

The position of the avian embryo on the yolk during early stages of development has a fairly definite relation to the axes of the egg. When an egg remains in a horizontal position for a few moments, the yolk comes to rest with the blastoderm on top. The embryo may then readily be observed by removing a portion of the shell above it. Embryos of the domestic fowl at 2 or 3 days of age were described by Von Baer (*1*) as lying at right angles to the long axis of the egg, with the head to the left when the small end of the egg is directed away from the observer. Early embryologists considered the normal orientation given by Von Baer to be relatively constant in all eggs, and used it in studies of segmentation as a means of predicting the future caudal and cephalic regions of the undifferentiated blastoderm. Later investigations have shown that marked deviations may occur from the normal orientation. Féré (*4*) measured orientations of a large number of chick embryos and found that 25 percent of them deviated by more than 45° to the right or left of the normal axis, This is in agreement with the findings of Dalton (*3*), Rabaud (*10*). Kopsch (*8*), and Taylor (*13*).

PROCEDURE

Since it was necessary not only to determine the orientation but also to give the embryo an opportunity to hatch, it was essential that the position of the embryo at an early age be determined without breaking the shell as had been done in most previous studies. Moreover, it was desired to obtain the readings of orientation as early as possible in the incubation process in order to ascertain more nearly the original orientation established during cleavage. For these reasons special apparatus had to be designed to permit observation of young embryos through the shell.

APPARATUS

A candling apparatus was constructed which contained two 500-watt projection lamps and an electric fan to cool them. The box was designed to give the maximum illumination of an egg placed in a

horizontal position in an egg-shaped hole in the top. Very little light could emerge except through the egg. The eggs of White Leghorn fowls were used, since their chalk-white shells transmit the light readily.

The top of the box was marked with lines which radiated out from the center of the egg-shaped hole every 15° throughout an entire circle. The line which represented the normal orientation of the embryo, being perpendicular to the long axis of the egg and 90° counterclockwise from the small end, was marked 0°. Other lines were marked 15°, 30°, 45°, etc., to the right or left of this line (fig. 2).

DETERMINATION OF ORIENTATION

The apparatus just described made readings of orientations possible at 3 days of incubation. The vitelline blood vessels of the normal embryo showed fairly well through the shell at 60 hours, and soon thereafter the body itself was visible. At 72 hours the cephalic and caudal regions could be distinguished, and in many cases the beats of the heart as well. Since the cranial and cervical flexures are at that age well advanced, the embryo itself is not entirely suitable for accurate measurements. It was found that the vitelline veins and arteries could be utilized along with the body of the embryo in determining the angle of orientation.

After about 72 hours of incubation the vitelline blood vessels were distinctly visible through the shell. These pass bilaterally from the embryo at right angles to its long axis and divide into two main branches, which extend in anterior and posterior directions. The forward branches develop more readily to form a wide fork, the arms of which are more or less parallel to the body of the embryo. The fork formed by the posterior branches occupies a similar relation to the embryo, but it is not so clearly defined until about 80 hours. By 90 hours, however, these vessels may have attained an equal or even greater length than those extending forward (fig. 1).

Since the object of the experiment was to determine the relationship between the definitive position just prior to hatching and the original orientation established in the early cleavage stages, it was desirable to determine that orientation at the earliest possible age consistent with accurate readings. After numerous trials this was found to be between 84 and 90 hours. At that time the embryo and the blood vessels both serve to differentiate the two ends of the body. The angle of orientation is indicated more exactly by the blood vessels than by the embryo itself, for the reason that they extend farther over the yolk and they are more plainly visible through the shell. The extra-embryonic blood vessels are less subject to changes of position than is the embryo, especially when the latter is enclosed within the amnion from the fourth day on. Moreover, it was found by breaking eggs at 48 hours that the branches of the blood vessels described above are almost exactly parallel to the straight line which the embryo's body then forms.

In eggs incubated in a horizontal position, the 4-day embryo is found on top of the yolk and near the highest point of the shell. Readings were quickly made in such eggs directly upon removal from the incubator. A number of the eggs used were incubated with the large end up, and the long axis of the egg about 45° from the vertical. In these some difficulty was at first encountered because, as the

eggs cooled, the embryonic membranes adhered to the inner shell membrane in the region of the air cell. This obstacle was easily overcome by placing all eggs in a horizontal position immediately upon their removal from the incubator, a procedure which caused the yolk to float freely so that the orientation of the embryo could be determined without difficulty. Readings were made to the nearest 15° radius.

DISTRIBUTION OF ORIENTATIONS

The orientations of 4,721 chick embryos were observed by candling on the fourth day of incubation. Of these 2,906 were in eggs incu-

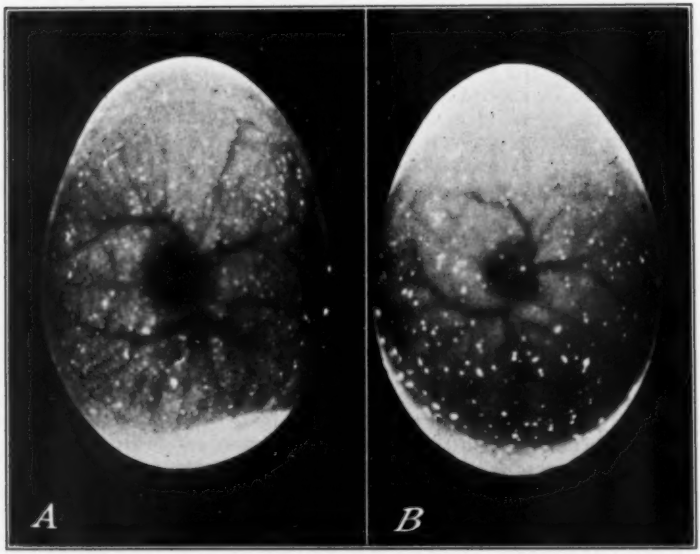

FIGURE 1.—Photographs of living chick embryos at 90 hours of incubation, taken through the shell of the egg. Owing to slight movements during the 10-minute exposures used in taking these pictures, the bodies of the embryos are less distinct here than when orientations were measured: *A*, Normal orientation; *B*, an embryo deviating to the right of the normal orientation. In both cases the anterior end of the embryo is to the observer's left.

bated in a horizontal position and 1,815 were in eggs incubated in the tilted position described above. Figure 2 shows the distribution of all these embryos at 24 different angles on the yolk.

The skewed distribution shown in figure 2 indicates that deviations occur more often to the right of the normal axis, i.e., toward the small end of the egg, than to the left. A similar condition is apparent also in the rather limited numbers reported by Dalton (*3*) and Kopsch (*8*). Unfortunately Féré's extensive data are not presented in sufficient detail to permit a comparison. Other data in this laboratory indicate that this skewed distribution of orientations arises from differences between individual hens with respect to the early orientations of their progeny.

The class with the center at 0° and a range of 7.5° on each side contains 1,491 embryos, or about one third of the total number.

Obviously the definition of normal orientation cannot be limited to such a narrow class. It has seemed preferable to consider as normal all orientations falling within the quadrant bounded by radii at 45° right and left of the so-called normal axis at 0° (fig. 2). The classes exactly at 45° right and 45° left contain 480 and 117 embryos, respectively. These cannot all be classed either as normal or abnormal, but by allotting them to the groups of normal and abnormal orientations in the proportion of 566:291 (right) and 243:76 (left)—the frequencies in the two adjacent classes of orientation—it was possible to determine the number belonging in the normal quadrant.

This permitted a comparison of the writers' determinations with those of Féré (*4*), Rabaud (*10*), and Kopsch (*8*), which is shown in table 1. It is evident from these data that with large numbers of chick embryos 75 percent of their orientations are to be expected within the normal quadrant.

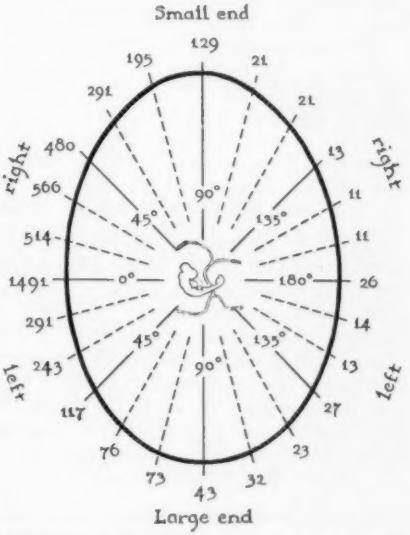

FIGURE 2.—Distribution of 4,721 chick embryos oriented at different angles with respect to the principal axes of the egg at 4 days of incubation.

TABLE 1.—*The proportion of normal orientations in the present data compared with those in other investigations*

Investigators	Method of observation	Total orientations	Orientations within normal quadrant
		Number	*Percent*
Cavers and Hutt	Through shell	4,721	74.4
Féré (*4*)	Shell removed	2,926	74.9
Kopsch (*8*)	do	155	62.6
Rabaud (*10*)	do	100	76.0

The accuracy of the method was tested by making a second measurement on 204 embryos 12 hours after the first. The eggs were turned in the incubator between readings, a procedure which would change the orientation if that were not relatively constant. The results, shown in table 2, indicate that the method used is remarkably accurate. Moreover, it has been shown (table 1) that the type of distribution is similar to that obtained by other workers. It is concluded therefore that the method of observing positions through the shell and using largely the vitelline blood vessels to indicate the angles of orientation gives essentially the same result as when the body is observed directly after breaking the egg. It has the special advantage of not interfering with the viability of the embryo.

TABLE 2.—*Comparison of two readings of orientation taken 12 hours apart on the same embryos, the eggs having been turned between readings*

Orientation	Eggs in indicated deviation class	
	Number	*Percent*
Same orientation at both readings	109	53.4
Deviation of 15°	80	39.2
Deviation of 30°	13	6.4
Deviation of 45°	2	1.0
Total	204	100.0

ORIENTATION AND SUBSEQUENT MORTALITY

In order that the dead embryos might be examined in good condition and the age at death estimated, the eggs were candled several times throughout the hatch. Of the 4,721 embryos whose orientations were measured on the fourth day, 3,864 were utilized in the study of subsequent mortality. The distribution of mortality among these during three subsequent periods of incubation and the total mortality are presented in table 3. The percentages of mortality in any one period e.g., 11 to 17 days, are based on the number of live embryos in each of the different classes of orientation at the beginning of that period. The percentages of total mortality are calculated on the original 3,864 embryos alive on the fourth day.

TABLE 3.—*Embryonic mortality at different periods of incubation in embryos originally having different orientations*

Orientation on fourth day (degrees)	Mortality in each period based on the number of embryos alive at the beginning of that period				Total mortality, 4–21 days	
	Embryos	4–10 days	11–17 days	18–21 days		
	Number	*Percent*	*Percent*	*Percent*	*Number*	*Percent*
135±22.5, left	54	5.6	0	5.9	6	11.1
90±22.5, left	131	4.6	4.8	20.1	36	27.5
45±22.5, left	357	3.9	4.7	17.1	86	24.2
0±22.5	1,971	5.0	5.0	15.5	467	23.7
45±22.5, right	1,002	7.5	5.7	17.3	279	28.0
90±22.5, right	271	5.5	5.9	20.3	79	29.2
135±22.5, right	38	7.9	2.9	14.7	9	23.7
180±22.5	40	2.5	7.7	16.7	10	25.0
Total or average	3,864	5.6	5.1	16.4	972	25.2

Of 3,461 embryos which survived to the nineteenth day, 222 were then removed and examined without being allowed to hatch. The expected 18-to-21-day mortality for these has been calculated to the nearest whole number in each class of orientation at the rate prevailing for that class in the same period among the remaining 3,239 embryos. For example, 26 of those removed had originally been oriented at 45° ± 22.5° left. The mortality after 18 days among 301 embryos originally so oriented, but given an opportunity to hatch, was 52, or 17.3 percent. Accordingly, the expected mortality for the 26 removed was 4 and the total 18-to-21-day mortality in the class was taken as 56. The number among which mortality was actually observed was so large that the calculation for the comparatively small number removed is quite justifiable.

For convenience, and to avoid fluctuations arising from small numbers, the embryos have been grouped in 8 classes of orientation. Each of these 8 includes 3 of the original 24 classes used when readings

were made. For example, the class at $45° \pm 22.5°$ right includes those embryos originally at 30°, 45°, or 60° to the right of the normal position, and, since these are mid-class values, the actual limits of the large class are at 22.5° right and left of the radius at 45° right.

The distribution of mortality is fairly uniform throughout the different classes of orientation except where the numbers are small to begin with. Embryos in the class at $135° \pm 22.5°$ left are especially favored, the rate of total deaths being less than half that found in the rest of the population. This might indicate an advantage to the embryo in being oriented toward the air cell of that egg were it not for the fact that in the class at $90° \pm 22.5°$ left, mortality is slightly in excess of that of the total population. High or low death rates in any of the classes with few embryos are accompanied by compensating fluctuations in adjacent classes and may therefore be attributed to chance. It is worthy of note, however, that the lowest total mortality (except in two classes containing small numbers) occurs in the modal class ($0° \pm 22.5°$), and that this class is the one containing embryos deviating not more than 22.5° from the orientation considered the norm for this species. A comparison of the mortality rate in this group with that for all other embryos reveals the following figures:

	Mortality (percent)
Normally oriented (1,971)	23.69 ± 0.97
All others (1,893)	26.67 ± 1.02
Difference	2.98 ± 1.40
Difference / Standard error	2.1

Since the difference between the mortality rates for these two groups is 2.1 times its standard error, it is statisticially significant, and since the rates for every class of abnormal orientation having adequate numbers are uniformly higher than that for the normal class, it may also safely be considered as biologically significant. This agrees with the finding of Taylor (*13*) that eggs oriented normally at 6 days hatched better than others by about 5 percent. The normal orientation is evidently slightly more conducive to the survival of the embryo than are deviations from it.

It is obvious that the excessive mortality among the abnormally oriented embryos occurs in the last 4 days of incubation and that it is to a large extent attributable to the comparatively high rate of 29.2 percent prevailing for embryos originally oriented at right angles to the normal orientation and with the head toward the small end of the egg ($90° \pm 22.5°$ right). The number in this class, however, is too small to indicate by statistical methods that its mortality rate is significantly higher than that for the normal class (23.7 percent) or for the entire population (25.2 percent). The excessive mortality in this class occurred chiefly in the last 4 days of incubation, and it will be shown later that it resulted from an excess of the head-in-small-end malposition.

The same class of orientation also exhibited high mortality during the period from 11 to 17 days. More extensive data would be necessary to prove that these embryos are under a handicap during this period, but such a possibility is indicated. Apart from this the mortality prior to the eighteenth day of incubation does not appear to be related to the original orientation of the embryo.

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ORIENTATION AND MALPOSITIONS

All eggs failing to hatch were examined on the twenty-second day. The positions were recorded of embryos which appeared to have survived beyond the eighteenth day of incubation, as indicated by size and by the amount of yolk enclosed within the body. Of 3,239 embryos which survived to the eighteenth day or beyond and which were given the opportunity to hatch, 533 failed to emerge from the shell. Some were alive and even pipped, but since they had not hatched on the twenty-second day they were considered as dead. Almost 65 percent of the 533 embryos were in one or other of the five malpositions described on page 517.

The early orientation of each embryo which died at 18 to 21 days is presented in table 4. Included with the frequency of each position at that age (o) is the expected frequency (c). The latter numbers were derived by allocating the embryos dying in a given position at 18 to 21 days to the various classes of orientation according to the proportions of the 3,239 embryos in those same classes at 4 days. If abnormal orientation has no relation to the occurrence of malpositions, there should be no significant difference between the observed and calculated distributions. The results of tests for goodness of fit are given in the two lower lines of the table. In making these calculations the classes having small numbers were combined, as is recommended by Fisher (*5*), to avoid the use of an expected number lower than 5. The values of P obtained for the distributions of positions normal, I, III, IV, and VI are well above the 0.05 level of significance, and differences between the observed and expected distributions of these positions may therefore be ascribed to chance. On the contrary, the distribution of position II gives a P value much smaller than 0.01, and the difference between the observed and expected values in this case is therefore highly significant. This means that more embryos in position II are associated with certain orientations than would be expected from chance alone.

TABLE 4.—*The early orientations of 533 fully formed embryos found dead in normal and abnormal positions at age of 18 to 21 days*

Orientation on fourth day (degrees)	Observed (o) and expected (c) frequencies of positions											
	Normal position		Position I		Position II		Position III		Position IV		Position VI	
	o	c	o	c	o	c	o	c	o	c	o	c
135±22.5, left	----	2.7	----	0.9	1	1.0	2	0.8	----	0.5	----	1.7
90±22.5, left	5	6.8	7	2.4	2	2.4	1	1.9	----	1.2	8	4.3
45±22.5, left	15	17.7	7	6.1	5	6.3	5	5.0	5	3.2	15	11.2
0±22.5	101	98.0	29	34.0	19	35.1	29	27.8	18	17.5	62	62.4
45±22.5, right	50	47.6	15	16.5	26	17.0	11	13.5	10	8.5	28	30.3
90±22.5, right	15	13.3	8	4.6	13	4.8	4	3.8	1	2.4	5	8.5
135±22.5, right	2	1.9	----	.7	1	.7	2	.6	----	.3	----	1.2
180±22.5	2	2.0	----	.7	1	.7	----	.6	----	.4	3	1.3
Total	190	190.0	66	65.9	68	68.0	54	54.0	34	34.0	121	120.9
χ^2	2.34		5.72		19.76		0.73		0.77		3.64	
P	.80		.22		<.01		.68		.68		.46	

The relation of abnormal orientation to the occurrence of position II is clearly demonstrated in table 4. The observed occurrences of this malposition are deficient in the 0° class and excessive in classes at 45°

and 90° right. It will be recalled that 90° right represents an orientation toward the small end of the egg and that similarly in malposition II the chick is upside down with its head in the small end of the egg (fig. 3, *B*). Abnormal orientation at an early stage is not the sole cause of position II, however, for 19 of the 68 embryos dying in that position at 18 to 21 days had been normally oriented on the fourth day, and 8 had actually been directed toward the large end of the egg.

The relation of abnormal orientations to subsequent positions of the embryo at hatching may also be measured in another way by considering what eventually happened to those originally having different orientations. The only limitation in such an analysis is that the positions of hatched chicks cannot be known with certainty and the analysis must be confined to those found dead at 18 to 21 days of incubation. Only the results for position II are presented

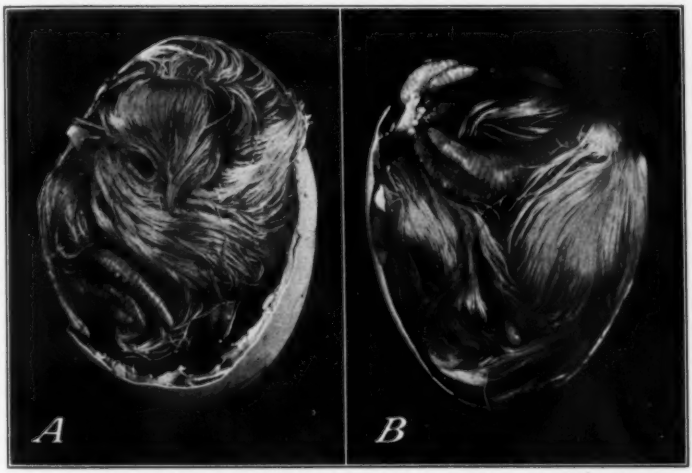

FIGURE 3.—Fully formed chicks which failed to hatch: *A*, Normal position, the head under the right wing and the beak just entering the air cell; *B*, position II, the embryo completely reversed with the head in the small end of the egg.

(table 5) because early orientation apparently had no relation whatever to the occurrence of the other malpositions. In table 5 the frequency of embryos dead in position II for each class of orientation is expressed as a percentage of the total number of embryos which were in that class on the fourth day and which survived to 18 days.

TABLE 5.—*Frequencies of position II in unhatched eggs, expressed as percentages of the 18-day live embryos in each class of original orientation*

Orientation on fourth day (degrees)	18-day embryos	Embryos dead in position II		Orientation on fourth day (degrees)	18-day embryos	Embryos dead in position II	
	Number	*Number*	*Percent*		*Number*	*Number*	*Percent*
135±22.5, left	46	1	2.2	90±22.5, right	227	13	5.7
90±22.5, left	116	2	1.7	135±22.5, right	33	1	3.0
45±22.5, left	301	5	1.7	180±22.5	35	1	2.9
0±22.5	1,670	19	1.1				
45±22.5, right	811	26	3.2	Total	3,239	68	2.1

The 68 cases of position II found among chicks failing to hatch represent 2.1 percent of all the embryos alive at 18 days. While the frequency of this malposition among the 1,670 embryos alive at 18 days and originally normally oriented ($0° \pm 22.5°$) was only 1.1 percent, its incidence among 1,569 similar embryos originally oriented otherwise was 3.1 percent, and among those originally oriented with the head toward the small end ($90° \pm 22.5°$ right) it attained the maximum frequency of 5.7 percent. This means that, apart from all other causes of mortality, the chance of dying in the head-in-small-end malposition during the last 4 days of incubation was, in the writers' material as a whole, over five times as great for the embryo directed toward the small end of the egg at 4 days of incubation as for the one not deviating more than 22.5° from the normal orientation at the same age. It will be shown later that this chance was lower among eggs incubated in one position than for those in another.

Taylor (*13*) has reported finding 50 percent more embryos in the head-in-small-end malposition at hatching time among those abnormally oriented at 6 days than among those normally oriented at the same age. From his abstract it would appear that all those not deviating more than 45° were considered as normal, and on this basis his results would seem comparable to those just given.

Further and somewhat more direct evidence of the influence of abnormal orientation upon the occurrence of malposition II is furnished by the data for 222 embryos which were all examined on the nineteenth day, and none of which were allowed to hatch (table 6). Position II occurred in 6.3 percent of these embryos. The numbers involved are small for the purpose of calculating percentages, but they do illustrate the marked tendency of embryos oriented toward the small end of the egg on the fourth day to be in a similar position at hatching.

TABLE 6.—*Occurrence of position II in embryos of known orientation, from eggs broken and examined on the nineteenth day*

Orientation on fourth day (degrees)	19-day embryos	Embryos in position II		Orientation on fourth day (degrees)	19-day embryos	Embryos in position II	
	Number	*Number*	*Percent*		*Number*	*Number*	*Percent*
135±22.5, left	5	1	20.0	90±22.5, right	14	5	35.7
90±22.5, left	3	0	0	135±22.5, right	1	0	0
45±22.5, left	26	0	0	180±22.5	1	0	0
0±22.5	109	4	3.7				
45±22.5, right	63	4	6.3	Total	222	14	6.3

The higher frequency of position II among these embryos than in those failing to hatch might be taken to indicate that a considerable proportion of chicks in this malposition are able to hatch were it not that the numbers involved are too small to permit a conclusion and that these eggs were all incubated in the horizontal position. As will be shown later, this position during incubation is particularly conducive to the assumption of the head-in-small-end malposition.

On the other hand, 9 of the 14 embryos originally oriented directly toward the small end of the egg (class 90° right, table 6), had succeeded in correcting their position before the nineteenth day. Of the

78 embryos oriented in the half of the egg away from the air cell (45° 90°, and 135° right) only 9, or 11.5 percent, retained that position. It is evident, therefore, that only a small proportion of these abnormal orientations have resulted in position II. Other malpositions in these same 222 eggs opened on the nineteenth day occurred independently of the early orientation.

REDUCTION OF THE RISK FOR ABNORMALLY ORIENTED EMBRYOS

In the fifth paper of this series Hutt and Pilkey (7) showed that position II is significantly more frequent in eggs incubated in a horizontal position than in those tilted at 45° with the large end up. The question naturally arises whether the tilted position makes it easier for all embryos to assume the correct position for hatching or whether it gains its advantage chiefly by facilitating the escape from the head-in-small-end malposition of the embryos originally oriented toward the small end of the egg. Analysis of the material upon which the present paper is based sheds some light upon this problem.

Of the 3,864 embryos of known orientation which were utilized in the study of subsequent mortality and malpositions, 2,049 were incubated in the horizontal position and the remainder in the tilted position, all being horizontal after the eighteenth day. In table 7 there is shown the effect of the tilted position upon the incidence of position II in embryos originally oriented (1) at $90° \pm 37.5°$ right and (2) any and all other ways including the normal orientaton at $0° \pm 25°$. The 222 embryos examined on the nineteenth day were deducted in determining the number of 18-day embryos. Accordingly, the figures record the incidence of chicks dead in position II among embryos alive at 18 days and given the opportunity to hatch. The inevitable slight error from the probability that some of those in that malposition had hatched is distributed equally to the two groups and therefore does not prejudice the data.

TABLE 7.—*Effect of the position of the incubating egg upon the incidence of position II in embryos originally oriented with the head toward the small end and in those oriented otherwise*

Position during incubation	Total embryos at 4 days	Embryos oriented in the given class		Embryos alive at 18 days	Embryos subsequently dead in position II		Difference between percentages dead in position II in horizontal and tilted positions
Embryos originally oriented 90°±37.5°, right:	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*	
Horizontal	2,049	274	13.4	210	19	9.05	7.14±2.19
Tilted	1,815	239	13.2	209	4	1.91	
Embryos in all other orientations:							
Horizontal	2,049	1,775	86.6	1,388	23	1.66	0.12±0.47
Tilted	1,815	1,576	86.8	1,432	22	1.54	

The percentages of the total number of embryos which were oriented toward the small end do not differ significantly in the horizontal and tilted eggs. This is to be expected if the orientation is established in early stages of cleavage. The fact that the expectation was realized indicates that the method used has permitted the

determination of the original orientation at a stage before it might be concealed by movement of the embryo or modified by the position of the incubating egg.

Among all the embryos not originally oriented within the sector bounded by radii at 37.5° right and left of the small end, there is no significant difference between the frequencies of position II in the horizontal and tilted eggs. These embryos constitute 87 percent of those alive at the eighteenth day and are sufficient in number to reveal even a small difference between the horizontal and tilted positions if there were one. On the other hand, among those originally oriented within 37.5° of the small end, the frequency of position II is nearly five times as great in the eggs incubated in a horizontal position as in the tilted ones. The difference—7.14 percent—is 3.3 times its standard error and therefore statistically significant even in the relatively small numbers available for comparison.

On the basis of this analysis and of the data in table 5 it seems safe to conclude that for all embryos surviving to 18 days there is some risk of subsequently dying in position II and that for about 87 percent of them that risk is only slightly lessened, if at all, by incubation in the tilted position. Table 5 indicates that this risk is smallest for those not originally deviating more than 15° from the normal orientation.

The chances of death in the head-in-small-end malposition after the eighteenth day were approximately as follows for embryos in the different classes of orientation at 4 days or earlier in the writers' material:

For embryos at $0° \pm 22.5°$ (normal), 1 per 100.
For other embryos, not within 37.5° of small end, 1.6 per 100.
For those at $90° \pm 37.5°$ right of normal, if tilted, 2 per 100.
For those at $90° \pm 37.5°$ right of normal, if horizontal, 9 per 100.

Hutt and Pilkey (*7*) pointed out that one way to reduce the mortality attributable to this malposition would be to incubate the eggs in the tilted position with the large end up during the critical period when the embryo is becoming fixed in its position with relation to the long axis of the egg. From the studies of Byerly and Olsen (*2*) this period would appear to be during the second week of incubation, while Kuo (*9*) terminates it by the eleventh day. The analysis given above shows why such treatment would be effective.

DISCUSSION

The frequent failure of chick embryos to attain the normal position for hatching undoubtedly accounts for the peak of mortality which occurs during the last few days of incubation. The head-in-small-end malposition (fig. 3, B), which has been shown in this study to result in some cases from incorrect orientation, places the embryo at a double disadvantage. With its head in the small end of the egg there is usually much less room for the embryo to work while initiating and carrying out the process of hatching. Moreover the air cell is inaccessible as a source of air to supplement the allantoic respiration before the shell is pipped.

Evidence has been gathered at this laboratory which shows that position II is likely to be fatal in the majority of cases, but on the other hand, a few chicks in this malposition have been definitely observed by the writers to hatch. It would appear, therefore, that though the

head-in-small-end position is usually fatal, it is not so invariably. This is not in accord with the opinion of Réaumur (*11*), who noted the malposition as early as 1751 but did not consider it a handicap.

Although position II seems to be of relatively infrequent occurrence when the total number of fertile eggs is considered, it accounts for no small portion of the mortality in eggs incubated in a horizontal position. Over 18 percent of 5,050 embryos dying on or after the eighteenth day in eggs so incubated were found by Hutt (*6*) to be in position II. From the data in table 7, which indicate that at least 19 out of 42 cases of position II in horizontal eggs resulted from abnormal orientation, it would appear that about 45 percent of such deaths, or about 8 percent of the mortality in the last 4 days of incubation, is traceable in horizontal eggs to abnormal orientations. This amounts to a little less than 4 percent of the total mortality. From the data in table 7 it would appear that for tilted eggs the mortality from this cause is considerably less than this figure.

Successive readings of orientation in the present study have shown that the extra-embryonic blood vessels and the yolk have a relatively constant relationship to the long axis of the egg from 72 to 96 hours, and that this relationship is maintained in most cases until the end of the sixth day. During this period the vitelline blood vessels serve to anchor the embryo in a fixed position upon the yolk, and the chalazae, in turn, maintain the yolk in a fairly constant relationship to the long axis of the egg. Kuo (*9*) points out that the restraint exercised upon the movements of the embryo by these two factors persists in varying degrees up to the tenth day of incubation. This is undoubtedly why some of the embryos originally directed toward the small end are never able to escape from that initial handicap. On the other hand, after the fifth day the embryo moves with increasing freedom up to the tenth day or slightly later (Kuo). This, in turn, explains why, in the writers' material, 98 percent of 209 embryos originally oriented within 37.5° of the small end were subsequently able to assume the normal head-in-large-end position in eggs incubated in the tilted position. Evidently the freedom of movement which permits such a shifting is somewhat more restricted in the eggs incubated in the horizontal position.

Further tests will be necessary to determine whether or not the occurrence of position II independently of the original orientation can be overcome by such manipulations as multiple turnings or turning the eggs in more than one plane during the critical second week when the head-in-small-end position is apparently established.

It is particularly interesting that the orientation associated with the lowest total mortality rate should also be that in which the greatest number of embryos is found (table 3). The rates 11.1 and 23.7 percent in embryos oriented at $135° \pm 22.5°$ left and $135° \pm 22.5°$ right are based on small numbers, and can therefore hardly be compared with the others which are based on over 100 embryos in each class. Apart from these two classes, the embryos not deviating more than 22.5° to right or left of the normal position have the lowest rate of mortality, one which was earlier shown to be significantly lower than that for all other orientations combined. The numbers in the five largest classes seem amply large to rule out the possibility of this being a coincidence, but further data bearing on this point are desirable.

The special interest lies in the probability that the orientation at right angles to the long axis of the egg, and with the head to the left when the small end of the egg is directed away from the observer, has become the normal and the modal orientation because of its survival value. Such an explanation is tenable only if the angle of orientation of the embryo is in some measure an inherited character. Obviously it is not a character of the embryo, but rather one that is maternally determined. If of two hens one should produce eggs containing embryos oriented within a very narrow range from $0° \pm 22.5°$, and another yield embryos with a much wider scatter of orientations, or within a narrow range but one deviating from the normal, the progeny of the first would have a slight advantage over that of the second. If the tendency exhibited by the first hen were inherited it would be preserved by natural selection and would in time become established as the normal for the species. Unpublished data collected by the writers support this hypothesis.

SUMMARY

The orientation of 4,721 chick embryos was accurately determined by candling, without breaking the shell, at 84 to 90 hours of incubation. Seventy-five percent of these embryos lay within 45° right or left of a line at right angles to the long axis of the egg and with their heads to the left when the small end of the egg was directed away from the observer.

The mortality rate for embryos originally normally oriented was significantly lower than that for all the remaining embryos.

Abnormal early orientation had apparently no markedly adverse effect upon the viability of the embryo until the last 4 days of incubation.

One malposition of the fully formed embryo, that in which the body is upside down with the head in the small end of the egg, was five times as frequent among embryos which had been oriented toward the small end of the egg on the fourth day as among those then normally oriented.

When eggs containing embryos originally oriented within 37.5° of the small end of the egg were incubated in a tilted position with the large end up, the frequency of the head-in-small-end malposition was reduced to 1.9 percent, but among such eggs incubated in a horizontal position its frequency was 9 percent. Tilting the eggs did not materially reduce the frequency of this malposition among embryos having other initial orientations.

Four other malpositions appeared to be independent of the original orientation of the embryo.

It is suggested that the normal orientation of the chick embryo has been evolved and established by virtue of the greater survival value which it confers upon the embryo.

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COLORADO AND VIRGINIA STRAINS OF CODLING MOTH IN RELATION TO THEIR ABILITY TO ENTER SPRAYED AND UNSPRAYED APPLES[1]

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INTRODUCTION

The amount of arsenical insecticide applied to control the codling moth (*Carpocapsa pomonella* L.) increased steadily in the leading apple-growing sections of the country until the amount of excessive spray residue on the fruit became a problem in the fall of 1925. Increased competition in the production of clean fruit and the concentration of orchards in certain areas, with the resulting increase in codling-moth population, have, doubtless, contributed to the necessity of increasing the number of sprays. The codling moth, however, continues as the major apple pest in spite of this. Just why this should be true is difficult to explain fully, but some of the possible factors involved are presented in this paper. The codling-moth material used in this study originated in two orchard districts having wide differences in requirements for control, namely, the Grand Valley of Colorado and the Shenandoah Valley of Virginia. A brief history of control requirements in each district is reviewed here.

An investigation of codling-moth control work in Virginia revealed that very few commercial apple orchards in the Shenandoah Valley were sprayed prior to 1900. According to pioneer growers the first orchard sprayer used in the Winchester district was soon after 1900. Up to 1915 the general practice was to apply one spray, the calyx application. In 1909 Quaintance and others (*10*)[3] demonstrated the efficiency of the calyx spray alone in controlling the codling moth in a number of orchards in the eastern part of the United States. Two of the orchards were in Virginia. From 1916 to 1920 the number of codling-moth sprays was increased to two, and in a very few instances to three, in the best-managed orchards. The author came to the Shenandoah Valley in 1921, a year when no sprays were applied because the apple crop was frozen. From 1922 to 1930 the number of applications was increased to four in the best-managed orchards. Because of economic conditions the number of sprays has not been increased since 1930.

A summary of the history of spraying for codling-moth control in the Grand Valley of Colorado was given to the writer by Dr. George List of the Colorado Agricultural Experiment Station in a letter dated December 8, 1928, as follows:

The first accurate record of the codling moth in the Grand Valley was in 1891. At that time the moth was just getting well established and was attracting some

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[3] Reference is made by number (italic) to Literature Cited, p. 552.

attention. A few of the growers began to spray about that time, but spraying was not general for some three or four years.

For a number of years only the calyx application was made. In 1900 and 1901 it was generally recommended that one cover spray be applied in addition to the calyx spray. In 1904 two cover sprays were quite generally used and a few of the growers were applying a third. During all of this time the percentage of loss was quite low, as compared with present losses. From 2 to 5 percent of wormy fruit was considered a very heavy loss. In 1906 the general recommendation was for five applications, the calyx and four cover sprays.

In 1907 Dr. Gillette and E. P. Taylor carried on an experiment in an orchard in that section in which they used a number of combinations. They came to the conclusion that four sprays were all that was necessary. In spite of this, however, the number of applications continued to increase. In 1909, which was the year of my first experience in that section, the general practice was to make five or six applications. By 1914 the infestation continued to increase, and six or seven sprays were used by practically all of the growers. From 1914 to the present time we have had demonstration blocks practically every year, and we have been testing the number of applications as well as a number of other points. In spite of all the information we have been able to get and to give the growers, the number of applications has been increasing and the results have not been as good generally as in former years. During the last four or five years the growers have been applying from eight to ten sprays and as high as twelve is not uncommon.

The wide difference in requirements for control is usually attributed to the influence of such environmental factors as climate and natural enemies, or to other local "conditions." It has been shown (*6*, *8*, *11*, *12*) that the seasonal history of the codling moth in the Grand Valley of Colorado and the Shenandoah Valley of Virginia is similar, even to the time of deposition of the various broods of eggs. A comparative study of the codling moth from the two valleys was undertaken at Winchester, Va., in 1927. It was hoped that information might be obtained concerning some of the reasons for the wide difference in requirements for control and in the number of sprays applied. Results of the first two season's work show (*4*, *5*) that the Colorado larvae were distinctly more successful in entering apples sprayed with lead arsenate and that crosses between the Colorado and Virginia moths were intermediate between the parent strains with respect to their ability to enter the sprayed fruit.

From 1929 to 1933 studies were included to determine whether the apparent tolerance is specific for arsenic, and to observe the effect of the Virginia climate on the Colorado strain and crosses, also the effect of rearing larvae on sprayed fruit exclusively, and something concerning the nature of the differences already demonstrated. Unless stated otherwise, the variety of apple used was the Arkansas (Mammoth Black Twig).

Thorpe (*13*) has reviewed the literature on the subject of insect strains or races.

METHODS

In comparing the ability of the larvae to attack sprayed fruit, the apples were sprayed by the writer in all instances. The calyx end of each apple was covered with shellac, and the apples were then suspended by their stems for spraying. Two sprays were applied, the second application being made as soon as the first coating had dried. Attempts were made to coat the apples alike in each test, and the residue was considerably greater with the double than with a single spraying. As soon as the spray material dried, codling-moth eggs were placed on the apples (average of 10 per apple) in the manner already described and illustrated by Hough (*5*). Eggs of the same

age were used for all strains in each experiment. The fruit was suspended in an insectary screened on all sides. The eggs hatched within 1 to 3 days after they were placed on the apples, and the fruit was examined 5 to 10 days after the eggs hatched.

Each year a fresh supply of lead arsenate was obtained for the experiments. The same commercial brand was used from 1927 to 1933, inclusive, except in 1929 when it was necessary to secure a fresh supply of another brand. The cryolite used in 1929 was a synthetic product whereas the cryolite used in 1933 was a natural product, processed for insecticide purposes.

Standard Weather Bureau maximum and minimum thermometers were used for recording temperature. A wet-and-dry-bulb hygrometer was used for humidity observations. Where larvae hatched in a saturated atmosphere the fruit was suspended in battery jars (gallon size), which contained about 2 inches of water. The top of each jar was closed with moisture-proof cellophane. When newly hatched larvae were confined in a saturated atmosphere the young worms were kept in closed Stender dishes containing a close-fitting disk of water-soaked blotting paper on the bottom.

A balance having a sensitivity of 0.01 milligram was used in weighing the larvae. One hundred newly hatched larvae were weighed at the same time. The larvae were collected in a straight-edged vial (one half inch in diameter and 3¼ inches high), a piece of cheesecloth was stretched over the open top and the vial placed in a cyanide bottle just long enough to render the larvae quiescent. This usually required about 20 minutes. Upon removal from the cyanide bottle and just before weighing, the larvae were carefully counted by placing one larva in each of 100 squares. When comparisons were to be made between strains, the work of collecting, preparing, and weighing each strain took place at the same time.

Granular potassium cyanide was used in preparing the cyanide bottles in which the newly hatched larvae were fumigated. Close-fitting disks of blotting paper kept the granules in the bottom. No water was added. Newly hatched larvae were collected in straight-edged vials as described above and fumigated for the desired period. With each fumigation the cyanide bottles were altered between the Colorado and Virginia larvae. In 1931 and 1932 pint fruit jars served as cyanide jars for fumigation of the eggs. In 1933 a large museum jar (35 by 30 centimeters) was used, so that all eggs of the various ages desired and of each strain could be fumigated simultaneously in the same chamber. To accomplish this, two circular wire baskets were made, each having six equal compartments. Twelve pear leaves or pieces of cellophane containing the eggs were placed in each of the 12 compartments, about one half inch of granular cyanide covered the bottom of the jar, and over the baskets there were four 4-inch Petri dishes filled with granular cyanide as shown in figure 1.

Second-instar larvae and mature larvae were fumigated in the museum jar just described. The larvae were first placed in Stender dishes, and each dish was covered with a fine-meshed wire gauze.

In the course of this investigation it was soon learned that dosages of insecticides (sprays or fumigants) could be made too great or too small to bring out any differences in the vitality of the individuals under test. When the dosage was too great, the kill approached 100 percent for all strains. When the dosage was too small, the proportion

of survival was so nearly the same for all strains as to mask any differences in their vitality. Correct dosage is absolutely essential in a comparative study of this kind.

DESIGNATION OF STRAINS

The Colorado strain consisted of larvae received October 24, 1927, from Grand Junction, Colo. Each generation was reared on unsprayed apples or on apples which carried only a very small amount of spray material.

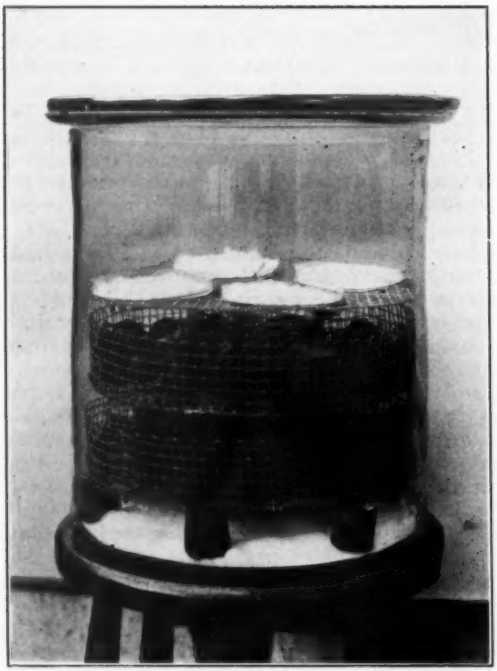

FIGURE 1.—Fumigation jar showing method of fumigating eggs on leaves placed in circular wire baskets of six compartments each.

The Colorado-K strain was received from Grand Junction November 12, 1928. Each generation was reared on apples sprayed with lead arsenate just before the fruit was placed in the rearing jars.

The Virginia strain was from native stock. Each generation was reared on unsprayed apples or on apples which carried only a very small amount of spray material. Larvae of this lot and the Colorado strain and their crosses were always reared on fruit which carried very little or no insecticide.

The Virginia-K strain also came from native stock. Each generation since July 1, 1929, was reared on apples sprayed with lead arsenate just before the fruit was placed in the rearing jars.

The Colorado-Virginia crosses were the progeny of crosses made with moths in July 1928. The F_1 generation was reared in 1928, the F_2 and F_3 generations in 1929, and so on, ending with F_{10} generation in June 1933.

The fruit used for the Colorado-K and Virginia-K larvae was first placed in a single layer on large wire trays (2 by 6 feet). A bucket sprayer was used to apply the lead-arsenate spray to all sides of the fruit until dripping began. For the Colorado-K larvae the fruit received lead arsenate at the rate of 1½ pounds per 100 gallons in the early years of the work, but this was finally increased to 4 pounds. For the Virginia-K larvae lead arsenate was used at the rate of 1 pound and finally increased to 2 pounds per 100 gallons.

EXPERIMENTAL RESULTS

STRAINS

At the close of the work in 1928 the question naturally arose as to whether the demonstrated difference between Colorado and Virginia larvae was specific only for arsenic. In 1929 such nonarsenicals as cryolite, barium fluosilicate, and rotenone were used in comparative tests with lead arsenate. The results (table 1) show that the Colorado larvae were distinctly more successful in entering fruit sprayed with any of the insecticides used. Of course, it was possible to use a material at such a strength that the difference was small as in the case of experiment 11 in which barium fluosilicate was used twice at the rate of 8 pounds per 100 gallons. Eggs placed on the apples sprayed with rotenone hatched 3 days after the spray was applied; and in the case of the other materials the eggs hatched within 1 to 3 days after the application of the sprays.

In experiment 7 the larvae designated as Colorado-K were from eggs deposited by the spring brood of moths, which came from larvae received from Grand Junction, Colo., on November 12, 1928. It appears that the Colorado-K and Colorado larvae were alike with respect to their ability to enter fruit sprayed with lead arsenate. Henceforth, the Colorado-K larvae were reared on fruit sprayed with lead arsenate a short time before the larvae hatched.

During the season of 1929 as well as in all succeeding seasons it was difficult to account completely for the differences in the percentage of control obtained. Temperature at the time of hatching of the larvae, variation in the spray covering, and the variety of apples used in different experiments may have contributed to the differences. Sometimes it seemed that the spray adhered to the fruit better than at other times, in spite of the fact that the applications were made as nearly alike as possible. Humidity or slight air movements at the time of spraying might have contributed somewhat, but the chief cause seemed to be the result of variations in the wax coating of the fruit. Markley and Sando showed (*9*) that the apple skin undergoes progressive changes in constituents and physical properties during growth. Ursolic acid and oil increase in amount, with oil increasing more rapidly as the apple approaches maturity. Furthermore, they showed that the apple skin on the sunny side contains larger quantities of oil than that on the shady side of the same fruit, while the opposite is true for ursolic acid content. In picking apples for the experiments reported herein no attention was given to the part of the tree from which the apples came. It follows that there must have been variations in the oil content of the skin of the fruit used.

In considering the factors influencing variations in control as recorded in table 1, the average number of eggs placed on each apple was 10 and the percentage of hatch was approximately the same for each strain in a given test. The number of apples used, however, was not always the same for each strain in a given test and the total number of apples used in each experiment was not always the same.

During the season of 1930 the lead arsenate tests were repeated in experiments 12 and 13 to learn whether any important change had taken place in the comparative ability of the Colorado and Virginia larvae to enter sprayed fruit. No important change was apparent in the results obtained. The results of similar experiments in 1932 and 1933 also failed to indicate any important change in either strain.

TABLE 1.—*Comparative tests with Colorado, Virginia, Colorado–K, and Virginia–K codling-moth larvae on apples which received 2 applications of an arsenical or a nonarsenical spray*

Experiment no.	Date	Apple variety	Strain	Check, unsprayed					Lead arsenate, 4 pounds in 100 gallons					Cryolite, 4 pounds in 100 gallons					Barium fluosilicate, 4 pounds in 100 gallons					Rotenone, 1 g in 2,000 ml				
				Eggs Hatched	Live larvae		Total injuries		Eggs Hatched	Live larvae		Total injuries		Eggs Hatched	Live larvae		Total injuries		Eggs Hatched	Live larvae		Total injuries		Eggs Hatched	Live larvae		Total injuries	
				No.	No.	Pct.	No.	Pct.	No.	No.	Pct.	No.	Pct.	No.	No.	Pct.	No.	Pct.	No.	No.	Pct.	No.	Pct.	No.	No.	Pct.	No.	Pct.
			Colorado	483	428	88.6	430	89.0	555	191	34.4	350	63.0	588	208	35.3	356	60.5										
7	May 25, 1929	Early harvest	Virginia	519	404	77.8	412	79.3	583	16	2.7	197	33.7	721	158	21.9	380	52.7										
			Colorado–K						565	191	33.8	348	61.5	436	152	34.8	293	67.2										
			Colorado	574	514	89.5	516	89.8	620	243	39.1	377	60.8	610	261	42.7	400	65.5										
8	June 6, 1929	Arkansas	Virginia	571	457	80.0	460	80.5	723	59	8.1	316	43.7	685	169	24.6	360	52.5										
			Colorado											°583	°169	°28.9	°278	°47.6										
			Virginia											°643	°108	°16.7	°270	°41.9										
			Colorado						486	125	25.7	243	50.0															
9	July 17, 1929	York imperial	Virginia	395	234	59.2	250	63.2	463	3	.6	74	15.9															
			Colorado	536	452	84.3	452	84.3	711	104	14.6	316	44.4	°634	°162	°25.5	°328	°51.7	459	46	10.0	185	40.3	377	57	15.1	67	17.7
11	Aug. 2, 1929	do	Virginia	605	390	64.4	388	64.1	803	2	.2	244	30.3	°656	° 44	° 6.7	° 236	°35.9	592	20	3.3	184	31.0	440	24	5.4	25	5.6
			Colorado																°559	° 24	°4.2	°209	°37.3					
			Virginia																°593	° 2	°0.3	°189	°31.8					
12	June 11, 1930	Arkansas	Colorado	405	351	86.7	361	89.1	582	119	20.4	307	52.7															
			Virginia	344	232	67.4	244	70.9	547	23	4.2	175	31.9															
13	Sept. 4, 1930	York imperial	Colorado						177	39	22.0	83	46.9															
			Virginia	357	265	74.2	273	76.4	364	11	3.0	78	21.4															
			Colorado	512	374	73.0	422	82.4	783	134	17.1	343	43.8															
18	June 25, 1932	Arkansas	Virginia	630	421	66.8	498	79.0	710	10	1.4	165	23.3															
			Colorado	200	150	75.0	155	77.5	348	63	18.1	153	43.9															
19	July 23, 1932	do	Virginia	230	125	54.3	135	58.6	319	8	2.5	75	23.5															
			Colorado–K						602	284	47.2	390	64.8															
			Virginia–K						380	62	16.3	137	36.0															
			Colorado	219	186	84.9	205	93.6	393	121	30.8	218	55.5															
20	Aug. 1, 1932	do	Virginia	245	159	64.9	193	78.8	325	4	1.2	61	18.7															
			Colorado–K						648	271	41.8	402	62.0															
			Virginia–K						460	88	19.1	228	49.5															
			Colorado	343	307	89.5	314	91.5	417	76	18.2	160	38.3															
21	June 7, 1933	do	Virginia	347	302	87.0	314	90.4	416	16	3.8	125	30.0															
			Colorado–K						389	116	29.8	194	49.8															
			Virginia–K						403	54	13.3	128	31.7															
			Colorado	405	359	88.6	364	89.8	439	133	30.2	268	61.0															
22	June 8, 1933	Baldwin (?)	Virginia	378	292	77.2	310	82.0	421	35	8.3	153	36.3															
			Colorado–K						866	328	37.8	492	56.8															
			Virginia–K						927	162	17.4	435	46.9															

| | | |

After rearing Colorado-K larvae on sprayed fruit through 7 generations and Virginia-K larvae through 6 generations, the eighth generation of Colorado-K and the seventh generation of Virginia-K larvae were included in tests made in July and August 1932. The results are given in experiments 19 and 20 (table 1). Colorado-K larvae were more efficient than Colorado larvae in their ability to enter and injure the fruit. A surprising difference was shown between Virginia-K and Virginia larvae, the former being distinctly more successful in entering the sprayed apples. Colorado larvae continued to demonstrate their superiority over Virginia larvae in their ability to enter sprayed fruit.

In the six experiments of 1933 (table 1) the order of success with which the larvae entered fruit sprayed with lead arsenate was Colorado-K, Colorado, Virginia-K, and lastly Virginia. The same order was also maintained in all tests with nonarsenical insecticides, except with nicotine in experiment 24 and cryolite in experiment 25. The fact that the Colorado-K strain did not exceed the Colorado strain in percentage of live larvae in experiment 25 might have been due to a slightly heavier coating of cryolite on the apples attacked by the Colorado-K larvae. In experiment 24 as well as in experiment 23 the heavy deposit of nicotine tended to obliterate marked differences between the strains. In these experiments the larvae hatched 2 days after the apples were sprayed with nicotine.

Three sets of crosses were made and the results of tests with the progeny of each were previously reported as shown in table 2. The first and second sets were discontinued after the F_2 generation of each cross was included in the tests of 1928. All subsequent experiments were made with the progeny of the third set of crosses and the results of these experiments are given in table 3.

TABLE 2.—*Crosses of the Colorado and Virginia codling moths*

Set of crosses	Crosses made	Previously reported tests with each generation			
		Experiment no.	Date of experiment	Generation	Reference [a]
First	June 1927	2	July 26, 1927	F_1	(4)
		3	Aug. 10, 1927	F_1	(4)
		4	June 8, 1928	F_2	(5)
		5	June 22, 1928	F_2	(5)
Second	May 1928	4	June 8, 1928	F_1	(5)
Third	July 1928	6	Aug. 9, 1928	F_2	(5)
		6	....do....	F_1	(5)

[a] Reference is to Literature Cited.

In 5 of the 8 tests recorded in table 3 the cross Colorado female × Virginia male was more successful in entering the sprayed fruit than was the cross Virginia female × Colorado male. If the totals of the 8 tests are considered, the results are as follows: Colorado female × Virgina male 3,942 eggs hatched, 368 live larvae or 9.3 percent; Virginia female × Colorado male, 4,202 eggs hatched, 330 live larvae or 7.8 percent. Both crosses maintained an intermediate position between the parent strains in the proportion of larvae which entered the fruit. Table 1 gives the results for the parent strains in the same experiments, which may be identified by the experiment number. Parent strains were not included in experiments 10 and 11a of 1929 and the Colorado strain was not included in experiment 15 of 1931.

TABLE 3.—*Comparative tests with the F_2 and succeeding generations of the crosses of the Colorado and Virginia codling moths on apples which received 2 applications of spray at the rate of 4 pounds of lead arsenate to 100 gallons of water*

Experiment no., date, and apple variety	Cross and generation	Eggs hatched	Live larvae		Total injuries	
		Number	*Number*	*Percent*	*Number*	*Percent*
9, July 17, 1929, York Imperial	Virginia ♀ × Colorado ♂ F_2	510	30	5.8	199	39.0
	Colorado ♀ × Virginia ♂ F_2	487	16	3.2	176	36.1
10, July 25, 1929, York Imperial	Virginia ♀ × Colorado ♂ F_2	556	40	7.1	208	37.4
	Colorado ♀ × Virginia ♂ F_2	570	53	9.2	229	39.7
11–a, Aug. 12, 1929, York Imperial	Virginia ♀ × Colorado ♂ F_2	485	14	2.8	140	28.8
	Colorado ♀ × Virginia ♂ F_2	503	30	5.9	149	29.6
12, June 11, 1930, York Imperial	Virginia ♀ × Colorado ♂ F_4	632	54	8.5	280	44.3
	Colorado ♀ × Virginia ♂ F_4	395	58	14.7	184	46.6
13, Sept. 4, 1930, York Imperial	Virginia ♀ × Colorado ♂ F_6	482	37	7.7	166	34.4
	Colorado ♀ × Virginia ♂ F_6	468	65	13.8	182	38.8
15, July 23, 1931, Arkansas	Virginia ♀ × Colorado ♂ F_7	701	33	4.7	283	40.4
	Colorado ♀ × Virginia ♂ F_7	637	48	7.5	272	42.7
21, June 7, 1933, Arkansas	Virginia ♀ × Colorado ♂ F_{10}	416	51	12.2	149	35.8
	Colorado ♀ × Virginia ♂ F_{10}	421	43	10.2	133	31.5
22, June 8, 1933, Baldwin (?)	Virginia ♀ × Colorado ♂ F_{10}	420	71	16.9	216	51.4
	Colorado ♀ × Virginia ♂ F_{10}	455	55	12.0	204	44.8

In all the work with the three sets of crosses 15 comparative tests were made from 1927 to 1933. In 7 of the experiments the cross of Virginia female × Colorado male showed a slightly higher percentage of live entrants, whereas the Colorado female × Virginia male was slightly more successful in 8 of the tests. Each cross showed a slightly higher percentage of live entrants in 2 of the 4 tests with larvae from the first set of crosses. The Virginia female × Colorado male was slightly more successful in both experiments which included larvae from the second set of crosses. In the third set, the Colorado female × Virginia male showed a higher percentage of live larvae in 6 of the 9 tests, 8 of which were made between 1929 and 1933 and are given in table 5. It is not clear that one cross was superior to the other in its ability to enter the sprayed fruit. It is clear, however, that both crosses were superior to Virginia larvae but inferior to Colorado larvae in this respect.

BACK CROSSES

In the spring of 1931 Colorado and Virginia moths of both sexes were crossed with moths of the F_5 generation of the cross Virginia female × Colorado male. Larvae of the F_1 and F_2 generations of back crosses were included in tests with Colorado and Virginia larvae and the F_7 generation of crosses. The results, given in table 4, show that crossing back with Colorado moths increased the ability of the larvae to enter sprayed fruit, while crossing back with Virginia moths did not. In fact, larvae of the F_2 generation of back crosses with Virginia cod-

ling moths were not so successful in entering sprayed fruit as were the crosses, and were most like Virginia larvae in this respect. It is probable that continued back crossing with the parent strains would have restored the Colorado and Virginia types.

In experiment 14, larvae of the back cross with Colorado males exceeded the Colorado strain in the percentage of live entrants. In the F_2 generation, however, the Colorado larvae were more successful. The back cross with Colorado females appeared slightly less successful in the F_1 generation than the back cross with Colorado males. In the F_2 generation, however, the back crosses were more nearly alike in their ability to enter the sprayed fruit.

In experiment 16 the percentage of live larvae was reduced for all larvae including the checks. It is thought that the chief cause of the reduction was the extra amount of lead arsenate on the fruit. The fruit was of the Arkansas variety as in the other tests, but the apples received an application of lead arsenate in the orchard about 3 weeks prior to the experiment.

TABLE 4.—*Comparative tests with Colorado and Virginia codling-moth larvae, the F_7 generation of crosses and F_1 and F_2 generations of back crosses on apples which received two applications of spray at the rate of 4 pounds of lead arsenate to 100 gallons of water, 1931*

	Experiment 14, June 13, F_1 of back cross			Experiment 15, July 23, F_2 of back cross			Experiment 16,[a] Aug. 3, F_2 of back cross		
	Eggs hatched	Live larvae		Eggs hatched	Live larvae		Eggs hatched	Live larvae	
BACK CROSS	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
Virginia ♀ × Colorado ♂ } ♀ F_1	547	129	23.6	829	117	14.1	394	31	7.9
Colorado ♂ × Colorado ♀									
Virginia ♀ × Colorado ♂ } ♀ F_1	469	80	17.0	849	109	12.9	439	35	8.0
Virginia ♀ × Colorado ♂ } ♀ F_1	553	38	6.9	862	18	2.0	386	5	1.3
Virginia ♂ × Virginia ♀									
Virginia ♀ × Colorado ♂ } ♂ F_1	473	35	7.4	913	31	3.4	455	8	1.7
STRAIN AND GENERATION OF CROSS									
Colorado	625	128	20.5				439	71	16.2
Virginia	499	9	1.8	645	12	1.9	433	3	.6
Virginia ♀ × Colorado ♂ } F_7				701	33	4.7	376	10	2.6
Colorado ♀ × Virginia ♂ } F_7				637	48	7.5	393	20	5.0
Colorado (check)							386	224	58.0
Virginia (check)							399	72	18.0

[a] Apples used in experiment 16 were sprayed by mistake with lead arsenate in the orchard during week of July 13.

SECOND-INSTAR LARVAE

Observations in June 1933 on the length of the first instar were made by placing 31 newly hatched Virginia larvae on thick slices of apples, which were kept in closed Stender dishes to conserve moisture. The larvae were removed soon after molting. The length of the first instar ranged from 3.5 to 7 days, averaging 4 days. Jenne (7) found the average length of the first instar to be 5.6 days (range 4 to 7 days)

for 12 individuals, but he concluded that handling the larvae daily or every 2 days in order to place them on fresh pieces of apple caused the larvae to develop slowly.

Colorado and Virginia larvae of the second instar ranging in age from 5 to 7 days were taken out of unsprayed fruit in the insectary and placed on sound apples. Although the second-instar larvae were not nearly so active as newly hatched larvae, table 5 shows slightly more than 60 percent of the Colorado larvae and a fraction more than 48 percent of the Virginia larvae were successful in entering unsprayed apples. On sprayed fruit, however, entry was very difficult, and it was necessary to reduce considerably the coverage of lead arsenate as compared with the coverage used for newly hatched worms in order to obtain a fair proportion of survival of both strains. The second-instar larvae were very sluggish when placed on sound apples and were inactive much of the time. Some fell from the fruit, others lost moisture and died, while others finally entered the apples. The Colorado larvae of the second instar appeared to be somewhat more successful in entering both sprayed and unsprayed fruit.

TABLE 5.—*Comparative tests[a] with Colorado and Virginia codling-moth larvae of the second instar on apples sprayed with lead arsenate, 1933*

Experiment no., date, and apple variety	Quantity of lead arsenate per 100 gallons	Strain of larvae	Age of larvae	Total larvae	Live larvae		Total injuries	
			Days	*Number*	*Number*	*Percent*	*Number*	*Percent*
1, June 29, Arkansas	2 pounds, 1 spray.	Colorado	6	300	64	21.3	74	24.6
		Virginia	6	300	30	10.0	52	17.3
	Check (not sprayed).	Colorado	5–6	200	126	63.0	130	65.0
		Virginia	5–6	200	97	48.5	104	52.0
2, June 30, Arkansas	1 pound, 1 spray.	Colorado	6–7	200	72	36.0	80	40.0
		Virginia	6–7	200	22	11.0	28	14.0
	Check (not sprayed).	Colorado	6	100	59	59.0	63	63.0
		Virginia	6	100	48	48.0	51	51.0
3, Aug. 7, Arkansas	3 pounds, 2 sprays.	Colorado	7	123	4	3.2	42	34.1
		Virginia	7	139	0	0	9	6.4
4, Aug 8–12, Arkansas	2 pounds, 1 spray.	Colorado	7	408	49	12.0	207	50.7
		Virginia	7	427	33	7.7	139	32.5

[a] In experiments 1 and 2 larvae were placed on the fruit suspended from poles in the usual manner. In experiments 3 and 4 larvae were placed on the apples in battery jars.

EFFECT OF TEMPERATURE ON LARVAE

In the early years of the work it was observed that a lower percentage of larvae usually entered the sprayed and unsprayed apples in an experiment, when the weather was cool during the period of hatching. Table 6 shows the relation between the temperature and the percentage of successful entrants found in unsprayed Arkansas apples. When the mean temperature ranged from 59° to 69° F. and the maximum from 70° to 82°, 62.4 percent of the Virginia larvae and 79.9 percent of the Colorado larvae entered the fruit; but when the mean temperature ranged from 70° to 82° and the maximum from 82° to 98°, the successful entrants increased to 76.3 percent for the Virginia larvae and 86 per cent for the Colorado larvae. A careful examination of percentage of live larvae in the sprayed fruit in the same experiments shows a somewhat similar effect. Cutright (*3*), using controlled temperatures, showed that the increase of temperatures within seasonal ranges aids the codling-moth larvae in establishing

themselves in sprayed and unsprayed apples. The data given in table 8 must be taken into consideration when accounting for variations obtained in the experimental results. Attention is also called to the consistently higher percentage of Colorado larvae as compared with the percentage of Virginia larvae found in the unsprayed apples in the various experiments.

TABLE 6.—*Relation between temperature and the number of successful entrants of Colorado and Virginia codling-moth larvae on unsprayed Arkansas apples which were used as checks in the various experimental tests*

Experiment no.	Year	Temperature during time of hatching		Virginia strain			Colorado strain		
		Mean	Maximum	Larvae	Live larvae		Larvae	Live larvae	
		°F.	°F.	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
4	1928	59	71	470	253	53.8			
17	1932	66	70	455	319	70.1	429	370	86.2
6	1928	67	77	480	295	61.4	438	369	84.2
5	1928	69	82	293	186	63.4	398	299	75.1
19	1932	69	76	230	125	54.3	200	150	75.0
24	1933	69	80	295	212	71.8	278	216	77.6
25	1933	69	80	290	179	61.7	308	236	76.6
Total				2,513	1,569	62.4	2,051	1,640	79.9
8	1929	70	90	571	457	80.0	574	514	89.5
27	1933	71	82	328	297	90.5	395	352	89.1
12	1930	72	85	344	232	67.4	405	351	86.7
26	1933	72	88	427	304	71.1	444	364	81.9
18	1932	78	89	270	196	72.6	216	173	80.0
20	1932	78	88	245	159	64.9	219	186	84.9
22	1933	80	98	378	292	77.2	405	359	88.6
23	1933	81	93	315	224	71.1	333	263	78.9
21	1933	82	98	347	302	87.0	343	307	89.5
Total				3,225	2,463	76.3	3,334	2,869	86.0

REJECTION OF POISONED MATERIAL BY LARVAE

Early in the investigation it was suggested that possibly the Colorado larvae rejected more of the poisoned part of the apple in entering the fruit. In 1930 a careful study was made of Colorado and Virginia larvae as they worked to enter the fruit. Two small pits about 1 millimeter square were cut side by side on the surface of an apple. A drop of a solution of arsenic acid was placed in each pit. In all but the last series the solution was stained with gentian violet to enable the observer to see where the poison had dried. A recently hatched Colorado larva was placed in one pit and a Virginia larva in the other. Both were viewed simultaneously in the same field of the binocular microscope.

In 20 minutes many of the larvae had buried themselves to such an extent that thereafter it was difficult to make accurate records of rejections. Others had quit on account of the action of the poison taken into the alimentary tract. The actual number of times each larva was seen to bite off a piece of apple pulp and throw it away was recorded. The totals for each series of observations and all observations are given in table 7. The larvae that did nothing in the first 20 minutes were not included. Three of these were Colorado and two Virginia larvae. None of the larvae was dead at the end of 20 minutes. A number appeared sick and had quit work, but it was

impossible to conclude from the number of rejections made in cutting through the poisoned walls of the apple pits which larvae were likely to die or live. There was no difficulty in seeing each rejection made following the bite cut loose from the apple. It appears that the poison each larva takes into its system is accidental and comes from adherence of minute amounts of poison to the mandibles. The poison may later be carried into the alimentary tract when the larva feeds. The larva's first concern is to bury itself and then feed, but in burying itself it cuts through and rejects the surface of the apple where the poison is found.

TABLE 7.—*Rejections of apple pulp made by newly hatched Colorado and Virginia codling-moth larvae during the first 20 minutes after being placed in pits poisoned with a solution of arsenic acid, 1930*

Series no.	Dilution of As_2O_5	Colorado strain						Virginia strain					
		Larvae—		Rejections in 20 minutes				Larvae—		Rejections in 20 minutes			
		Alive	Dead	Maximum	Minimum	Total	Average	Alive	Dead	Maximum	Minimum	Total	Average
1	1 to 5,000	10	0	42	3	175	17.5	7	1	33	1	110	15.7
												31	31.0
2	1 to 1,000	7	2	29	2	112	16.0	6	4	35	1	76	12.6
				19	5	24	12.0			21	9	64	16.0
3	do	9	1	49	14	261	29.0	5	5	44	6	129	25.8
						16	16.0			33	3	100	20.0
4	do	8	2	52	12	214	26.7	8	2	45	7	229	28.6
				20	7	27	13.5			31	8	39	19.5
5	do	6	3	70	4	185	30.8	5	5	63	10	176	35.2
				33	14	62	20.6			43	14	123	24.6
6	do	5	4	33	8	85	17.0	2	8	27	2	29	14.5
				11	6	36	9.0			50	7	186	23.2
Total (alive)		45				1,032	22.9	33				749	22.6
Total (dead)			12			165	13.7		25			543	21.7
Percent		78.9	21.1					56.9	43.1				

Table 7 shows that the 12 Colorado larvae which finally died averaged 13.7 rejections per larva as compared with 21.7 rejections per larva for the 25 dead Virginia larvae. In other words, the Colorado larvae were fatally poisoned after biting off a fewer number of pieces and rejecting them, but only 21 percent of the Colorado larvae died as compared with 43.1 percent of the Virginia larvae. The average number of rejections per larva made by the live larvae was practically the same for both strains.

WEIGHT OF LARVAE

Campbell (*1*) working with silkworms showed that the minimum lethal dose of arsenic varied directly with the weight of the insect and could be expressed per unit weight or mass of insect. It was thought that a comparison of the weights of young codling-moth larvae of the various strains might yield some information that would aid in explaining the observed differences in the ability of the larvae to enter sprayed and unsprayed apples. By weighing 100 larvae at a time it was found (table 8, series A, B, C) that all the larvae weighed distinctly less when the temperature was high and the humidity relatively low as in series B. In series A and C the percentage of

atmospheric humidity was about the same for each series, but the saturation deficiency of the air and, therefore, the evaporation was probably greater for series C, because the temperature was higher. It will be observed the larvae of each strain in series C weighed slightly less than those of the corresponding strain in series A. Virginia larvae under atmospheric conditions weighed less than Colorado larvae, but the weight of the cross Virginia female × Colorado male in series A exceeded the weight of the Colorado larvae, and in series B, the cross Colorado female × Virginia male was equal in average weight to the Colorado larvae. In other words, there was no evidence of any relation between weight of larvae and the comparative ability of the strains and the crosses to enter sprayed fruit.

TABLE 8.—*Weight of young codling-moth larvae of the Colorado and Virginia strains as determined by weighing 100 larvae at each weighing*

Series	Date	Temperature [a]	Humidity [a]	Strain	Age of larvae	Weighings	Total larvae weighed	Total weight	Average weight—	
									100 larvae	Each
		°F.	*Per cent*		*Hours*	*Number*	*Number*	*Milligrams*	*Milligrams*	*Milligrams*
A	May 21 to June 3, 1930.	73.0	60	Colorado	Up to 1	10	1,000	44.41	4.44	0.0444
				Virginia	do	19	1,900	82.80	4.35	.0435
				Virginia ♀ × Colorado ♂ F_1	do	10	1,000	45.05	4.50	.0450
				Colorado ♂ × Virginia ♀ F_1	do	10	1,000	43.41	4.34	.0434
				Colorado	do	18	1,800	70.68	3.92	.0392
				Virginia	do	17	1,700	63.22	3.71	.0371
B	July 21–28, 1930.	91.7	44	Virginia ♀ × Colorado ♂ F_1	do	5	500	18.27	3.65	.0365
				Colorado ♀ × Virginia ♂ F_1	do	5	500	19.61	3.92	.0392
C	June 2, 1932	80.8	63	Colorado	do	5	500	22.11	4.42	.0442
				Virginia	do	5	500	20.56	4.11	.0411
D	June 3–23, 1932.	74.7	100	Colorado	1½ to 2½	12	1,200	57.19	4.76	.0476
				Virginia	do	12	1,200	57.22	4.76	.0476
			72	Colorado	do	12	1,200	50.94	4.24	.0424
				Virginia	do	12	1,200	49.35	4.11	.0411

[a] Average at time of collecting and weighing.

In series D of table 8 is found the weight of newly hatched Colorado and Virginia larvae which were kept in a saturated atmosphere, compared with the weight of larvae kept at atmospheric humidity for the same length of time. There was no significant difference in the weight of the Colorado and Virginia larvae in the saturated atmosphere. The Colorado larvae under atmospheric conditions weighed slightly more than the Virginia larvae, and both strains weighed distinctly less than the larvae kept in a saturated atmosphere. The weight of the Colorado larvae under atmospheric conditions was 10.9 percent less and that of the Virginia larvae 13.7 percent less than the weight of the corresponding strain kept in a saturated atmosphere at the same time.

In this connection it should be stated that the weights of full-fed or mature larvae of the Colorado and Virginia strains were found to be nearly the same when weighed from September 11 to 16, 1929. In 10 weighings of 10 larvae each, the Colorado larvae slightly exceeded the Virginia larvae in 5 of the trials and were exceeded in weight by the Virginia larvae in the other 5 trials. The total weight

of 100 full-fed Colorado larvae was 5,503.06 milligrams, as compared with 5,529.21 milligrams for 100 full-fed Virginia larvae. That is, the average weight of a full-fed Colorado larva was 55.03 milligrams and that of a Virginia larva 55.29.

ENDURANCE OF STARVATION

Newly hatched larvae were confined in Stender dishes for periods varying from 24 to 28 hours. The larvae were then examined and all individuals that showed signs of life when disturbed with a needle were included with the live larvae. Larvae of the strains to be starved were collected at the same time and confined in Stender dishes. For this work it was necessary to use a dish having a cover ground very accurately to fit, in order to prevent the escape of an occasional larva. Results of the tests are given in table 9. The Colorado larvae exhibited greater endurance to starvation than Virginia larvae in all 3 years in which comparisons were made. It is apparent that the number of larvae that survived increased as the temperature during the period of starvation was lowered. Little difference in endurance was shown between the Colorado and Colorado-K larvae in 1933. There was also little or no difference between Virginia and Virginia-K larvae, except in the tests during the cool weather of August 21, 1933, when the Virginia-K larvae survived in about the same proportion as the Colorado larvae.

TABLE 9.—*Endurance of starvation of young codling-moth larvae of the various strains*

Year	Date	Period of starvation	Temperature		Colorado strain			Virginia strain			Colorado-K strain			Virginia-K strain		
			Range	Mean	Larvae	Live larvae		Larvae	Live larvae		Larvae	Live larvae		Larvae	Live larvae	
		Hours	° *F.*	° *F.*	*No.*	*No.*	*Pct.*	*No.*	*No.*	*Pct.*	*No.*	*No.*	*Pct.*	*No.*	*No.*	*Pct.*
1930	July 24	26 to 28	67–95	81	264	41	15.5	500	31	6.2						
1931	July 17	do	69–89	79	173	49		139	26							
	Aug. 10	do	70–89	79½	279	44		309	45							
	Aug. 16	do	66–88	77	248	31		276	13							
		Total			700	124	17.7	724	84	11.6						
1933	Aug. 6	24 to 26	59–83	71	302	105		447	108							
	Aug. 10	do	64–78	71	257	123		461	159		431	151		306	93	
	Aug. 12	do	64–90	77	287	49		398	62		108	23		181	37	
	Aug. 18	do	67–87	77	98	20		150	28		144	28		129	28	
	Aug. 19	do	66–86	76				108	30		198	63		255	62	
		Total			944	297	31.4	1,564	387	24.7	881	265	30.0	871	220	25.2
	Aug. 21	24 to 26	62–73	67½	343	214	62.3	355	174	49.0	241	161	66.8	316	195	61.7

The average weight of the larvae that survived the starvation tests of July 30, 1930, was 0.024 milligram per larva for the Colorado strain and 0.019 milligram for the Virginia strain. A comparison of the average weight of a starved larva with the average weight of a recently hatched larva of the same strain as determined July 21 to 28, 1930 (table 8, series B), shows that during the period of starvation the average reduction in weight per larva was 0.015 milligram, or 38.4 percent for the Colorado strain, and 0.018 milligram or 48.6 percent for the Virginia strain. In other words, loss in weight was less for the Colorado larvae than for the Virginia larvae. This was also

demonstrated in the comparison of weights made in series D (table 8). Loss in weight may result from hunger and loss in moisture. In the case of series D, the latter is probably the chief cause. Difference in the rate of loss of moisture may be due to a difference in water-binding colloids in the tissues of the larvae rather than to any difference in the structure of the epidermis.

FUMIGATION TESTS

Eggs

Comparative tests were made in which codling-moth eggs of various strains were fumigated with potassium cyanide. Two experiments in 1931 and three each in 1932 and 1933 were made, in which the eggs were fumigated for a period of 2 hours. During the season of 1933 there were eight fumigation tests, each for a period of 1½ hours. In each test in 1933 all eggs were fumigated at the same time in the same fumigation chamber; in the other years individual fumigation jars were used at the same time. The results are recorded in table 10. Eggs of three ages or stages of embryonic development were fumigated at the same time; in the first stage were eggs less than 24 hours old, mostly between 12 and 16 hours; in the second stage eggs intermediate between recently deposited eggs and eggs nearly ready to hatch; and in the third stage eggs due to hatch within 1 to 12 hours after they were placed in the fumigation chamber.

TABLE 10.—*Summary of experiments on the comparative tolerance of Colorado, Colorado–K, Virginia, and Virginia–K codling-moth eggs to fumigation with potassium cyanide*

Year	Duration of fumigation	Age of eggs	Colorado eggs			Virginia eggs			Colorado–K eggs			Virginia–K eggs		
			Total	Hatched		Total	Hatched		Total	Hatched		Total	Hatched	
	Hours	*Hours*	*Number*	*Number*	*Per cent*	*Number*	*Number*	*Per cent*	*Number*	*Number*	*Per cent*	*Number*	*Number*	*Per cent*
1933	1½	2–24	3,399	1,881	55.3	3,345	1,974	59.0	3,550	1,822	51.3	3,030	1,628	53.7
		60–84	2,191	1,068	48.7	1,801	559	31.0	2,085	1,157	55.4	1,752	627	35.7
		(a)	6,459	3,350	51.8	5,408	816	15.0	4,455	2,636	59.1	3,554	871	24.5
	2	2–24	1,337	602	45.0	1,344	793	59.0	1,774	731	41.2	1,709	794	46.4
		60–72	932	519	55.6	752	253	33.6	805	564	70.0	926	548	59.1
		(a)	1,571	179	11.3	1,734	4	.2	1,493	405	27.1	1,343	52	3.8
1932	2	2–24	310	161	51.9	1,001	519	51.8	.....	.....	.....	.....	.....	.....
1931	2	2–24	606	350	57.7	1,031	487	47.2	.....	.....	.....	.....	.....	.....
		(a)	596	341	57.2	1,472	77	5.2	.....	.....	.....	.....	.....	.....

a Eggs mostly 5 to 6 days old; all over 100 hours old. The eggs were due to hatch within a few hours when fumigated.

There was no indication of a significant difference in tolerance to fumigation between recently deposited eggs of the different strains. On the other hand, there was a marked difference in the tolerance of eggs almost ready to hatch. The order of tolerance was Colorado–K, Colorado, Virginia–K, and Virginia. This is the same order as that established by young larvae of the same strains in entering sprayed apples. Embryonic development was slowed down greatly in the fumigated eggs. Often an embryo developed into an apparently normal larva which perished without having sufficient strength to break through the chorion. In 1931 the temperature was an average of 7° lower at the time of the 2-hour fumigation tests than in 1933.

The difference in temperature and the fact that different fumigation chambers were used must be taken into consideration in comparing the results obtained in the experiments of the 2 years. In 1932 the temperature at the time of fumigation was about the same as the temperature in 1931.

Cotton (*2*) has shown that the susceptibility of an insect to a fumigant varies directly with the rate of respiratory metabolism. This explains why eggs almost ready to hatch were more susceptible to potassium-cyanide fumigation than recently deposited eggs, in which the respiratory rate is low. It will be observed, however, that the Colorado and Colorado–K eggs in the 1½ hours of fumigation in 1933 and the Colorado eggs in the 2 hours of fumigation in 1931 showed little or no decrease in the percentage of hatch of the eggs containing well-developed embryos as compared with the percentage of hatch for the youngest eggs fumigated at the same time. In these tests the majority of well-developed embryos in the Colorado and Colorado–K eggs were not carried beyond the critical stage for recovery, so that hatching occurred later; but the critical stage was passed in most of the eggs of the same strains and embryonic development in the 2 hours of fumigation in 1933. It is apparent that eggs of the different strains containing embryos in the late stages of development have different levels for the critical stage beyond which recovery is impossible. In other words, a difference in vigor or hardiness (that is, power of recovery) becomes apparent as the embryo develops and is most marked when the embryo is fully developed. The percentage of eggs having a high level of vigor or greater power of recovery differed with each strain. The greatest percentage was in the Colorado–K strain, a close second was the Colorado strain, next in order was Virginia–K, and lastly, Virginia.

Young Larvae

Newly hatched Colorado and Virginia larvae were fumigated with potassium cyanide and then placed on whole apples or on slices of apples and kept in Stender dishes for 24 hours. A count was then made of the dead and live larvae. The results are given in table 11.

Table 11.—*Summary of experiments on the comparative tolerance of newly hatched Colorado and Virginia codling-moth larvae to fumigation with potassium cyanide*

Year	Experiments	Fumigation period	Food provided larvae after fumigation	Colorado strain			Virginia strain		
				Larvae fumigated	Larvae alive 24 hours after fumigation		Larvae fumigated	Larvae alive 24 hours after fumigation	
	Number	*Hours*		*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
1931	9	1	Whole apples	444	142	31.98	534	78	14.6
	3	1	Sliced apples	141	92	65.24	135	39	28.8
	16	2	Whole apples	753	148	19.65	908	50	5.5
	9	2	Sliced apples	393	162	41.22	428	90	21.0
1932	13	2	do	1,847	615	33.29	2,424	396	16.3
1933	21	2	do	2,101	639	30.41	2,172	355	16.3
1931	4	(a)	Whole apples	183	132	72.13	219	118	53.8
	5	(a)	Sliced apples	209	185	88.51	211	176	83.4

a Not fumigated.

It is apparent that the Virginia larvae were more susceptible to potassium-cyanide fumigation than the Colorado larvae. There was a

higher percentage of live larvae of each strain when the fumigated larvae were placed on sliced apples after fumigation than when placed on whole apples. Many of the larvae on whole apples did not recover sufficiently to enter the fruit and died from starvation. In short, the power of recovery was possessed to a greater degree by more of the Colorado larvae than the Virginia larvae. It is reasonable to assume that the power of recovery is an index of the vigor inherent in the larvae. A similar but less marked difference was demonstrated by the check experiments.

Larvae of Second and Last Instars

Colorado and Virginia larvae of the second instar (7 days old) were removed from apples and fumigated with potassium cyanide. The fumigated larvae were then placed on slices of apples in Stender dishes and examined 24 hours later. The results are shown in table 12. A higher percentage of Colorado larvae survived the fumigation tests. The check experiments also showed a higher percentage of Colorado larvae able to survive being transferred from their natural environment in the apple to slices of apples in Stender dishes.

Table 12.—*Summary of experiments on the comparative tolerance of second-instar and also of mature or full-fed larvae of the Colorado and Virginia codling-moth strains to fumigation with potassium cyanide, 1933*

Larval stage	Experiments	Fumigation period	Colorado strain			Virginia strain		
			Larvae fumigated	Larvae alive 24 hours after fumigation [a]		Larvae fumigated	Larvae alive 24 hours after fumigation [a]	
	Number	*Minutes*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
Second instar	3	45	77	54	70.1	77	45	58.4
	1	60	24	16	66.6	25	10	40.0
	8	75	193	72	37.3	208	46	22.1
	4	[b]	92	82	89.1	93	70	75.2
Last instar	12	300–420	1,516	857	56.53	1,537	713	46.38

[a] Second-instar larvae were placed on sliced apples after fumigation and kept in Stender dishes.
[b] Not fumigated.

Full-fed larvae of the two strains were also fumigated with potassium cyanide and then kept in museum jars for 24 hours before they were examined. All larvae showing a trace of life when disturbed with a needle were considered alive. In all tests the larvae were fumigated in the same jar at the same time. The results (table 12) show a slight difference in survival in favor of the Colorado larvae. The difference does not appear marked as shown by larvae of the first instar or by the eggs containing embroyos in the late stage of development.

SUMMARY AND CONCLUSIONS

The history of the codling moth in the Grand Valley of Colorado and the Shenandoah Valley of Virginia shows that the insect has been much more difficult to control in Colorado. The seasonal history of the codling moth is essentially alike in the two districts, according to published records.

Larvae from Colorado and native Virginia larvae have been reared and studied under the same climatic environment in Virginia for 7

years. This paper reports results of the investigations of the past 5 years.

Colorado larvae reared under Virginia climatic conditions since 1928 have consistently demonstrated a distinct superiority over Virginia larvae in their ability to enter sprayed fruit.

Greater ability of the Colorado larvae to enter sprayed fruit was not specific for lead arsenate but was also demonstrated when such nonarsenical sprays were used as cryolite, barium fluosilicate, rotenone, cuprous cyanide, and nicotine.

From 1929 to 1933 larvae of Colorado origin and native Virginia larvae were reared on fruit freshly sprayed with lead arsenate. These larvae were designated as Colorado–K and Virginia–K strains, respectively, to distinguish them from the Colorado and Virginia larvae reared on unsprayed apples or on fruit carrying a very small amount of lead-arsenate residue.

In 1933 the Colorado-K, Colorado, Virginia-K, and Virginia larvae demonstrated their comparative ability to enter sprayed fruit in the order named, that is, the Colorado-K larvae were most successful and the Virginia larvae least successful in all tests regardless of the kind of insecticide used. Comparative tests were made on fruit sprayed with cryolite, cuprous cyanide, nicotine, and lead arsenate.

Colorado-Virginia crosses, through 10 generations, maintained an intermediate position between the parent strains with respect to their ability to enter fruit sprayed with lead arsenate.

Back crosses between the F_5 generation of the cross Virginia female × Colorado male and Colorado moths demonstrated a marked increase in ability to enter sprayed fruit, while the back cross with Virginia codling moths showed a tendency to approach the Virginia strain in ability to enter the sprayed fruit.

Colorado larvae of the second instar were slightly superior to Virginia larvae in ability to enter unsprayed and sprayed fruit.

Larvae of Colorado and Virginia strains were more successful in entering unsprayed fruit as well as sprayed fruit when the temperature was relatively high than in cooler weather.

Larvae of the Colorado strain under all conditions were consistently more successful than Virginia larvae in entering unsprayed fruit.

Careful observations on the habit of rejecting apple tissue while entering the fruit failed to reveal any difference in habit of entering that could be used to explain the difference in survival of the Colorado and Virginia larvae under observation. By actual count the number of rejections made by the Colorado larvae did not exceed those made by the Virginia larvae.

At atmospheric temperature and humidity, the average weight of recently hatched Colorado larvae was slightly greater than that of Virginia larvae. Recently hatched larvae in a saturated atmosphere weighed the same for both strains. Under atmospheric conditions the average weight of recently hatched larvae of the Colorado-Virginia crosses varied from slightly less than the average weight of Virginia larvae to slightly more than the average weight of Colorado larvae.

Colorado larvae endured starvation more successfully than Virginia larvae. The percentage of survival increased for both strains when the larvae were starved during cool weather in comparison with survival when the temperature was relatively high.

Loss in weight of starved larvae was greater for Virginia larvae than for Colorado larvae. Difference in the weights of Colorado and Virginia larvae under atmospheric conditions may be due to loss of moisture. The rate of loss of weight between the strains may be due, theoretically, to a difference in water-binding colloids in the tissues of the larvae rather than to any structural difference in the epidermis.

There was little or no difference in tolerance to potassium cyanide fumigation between recently deposited eggs (less than 24 hours old) of the four strains.

There was a distinct difference in tolerance to potassium cyanide fumigation of eggs of the four strains when the eggs contained embryos in the late stages of development. The percentage of hatch was greatest for Colorado-K, then Colorado, next Virginia-K, and lastly Virginia eggs.

There was a progressive increase in susceptibility to the fumigant as the embryo developed in the eggs of all strains but the increase in susceptibility was greatest for Virginia eggs and least for Colorado-K eggs.

Newly hatched larvae of the four strains showed the same order of tolerance as did the eggs with well-developed embryos when subjected to potassium cyanide fumigation.

The essential difference in the strains was demonstrated in the partially developed embryo, was most marked in the fully developed embryo and the newly hatched larvae, and disappeared to a large extent in the full-fed larvae. The difference seems to be one of general vigor, or power of recovery, inherent in the individual.

This investigation demonstrates the existence of different strains of the codling moth in which the young larvae vary greatly in vigor. By rearing the larvae continuously on freshly sprayed fruit in the laboratory, it was possible to increase in a strain the proportion of individuals which possessed more vigor, with the result that a greater percentage of the young larvae entered and injured sprayed apples. Difference in vigor appeared in the well-developed embryo and was most marked in the fully developed embryo and in newly hatched larvae. It was also quite evident in larvae of the second instar but had disappeared to a large extent in the full-fed or mature larvae.

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THE NUTRITIVE VALUE OF THE PROTEINS OF ALFALFA HAY AND CLOVER HAY WHEN FED ALONE AND IN COMBINATION WITH THE PROTEINS OF CORN[1]

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INTRODUCTION

The great importance of legume hay is recognized by everyone interested in livestock production. Because of this importance, it is highly desirable to obtain data on the nutritive value of the proteins in these hays when they are fed alone and when fed in combination with the protein of corn and other farm-grown grains.

It is apparent that the great value of legume hays in stock feeding is largely due to their heavy yield per acre, their high protein content, and their palatability. From the good results which are obtained with legume hay, even when animals are given little or no other feeds in addition, as is sometimes the case in the alfalfa districts of the West, it might also be assumed that the protein of legume hay has a high quality or high biological value. The results of experiments thus far reported, however, do not seem to bear out this assumption. Practically all experiments in which alfalfa hay furnished the principal or sole source of protein have indicated that the crude protein possesses a low nutritive value. Sotola (*20*)[2] found the protein of alfalfa hay to have a biological value of only 56 when fed alone to lambs. This was considerably lower than values for corn silage or even for sunflower silage. A later report by Sotola (*21*) indicated still lower biological values for alfalfa protein when fed to lambs. This report gave the protein of the alfalfa stems a value of 64, while for the protein in the leaves it was only 44 and for the protein in the whole hay it was 51.

Haag (*3*) made a study of the factors limiting the nutritive value of the crude protein of alfalfa for rats, and reported a deficiency of cystine, one of the essential amino acids, in the proteins of this hay. Nevens (*17*) fed alfalfa to rats at a 10 percent level of intake and obtained an average biological value of 62. His results further indicated no supplementary effect of the corn-grain proteins with alfalfa proteins, since the combination gave biological values averaging 58.

Very few data reported show any marked differences in efficiency of protein utilization when common feeding stuffs have been fed to ruminants. Experiments by Hart, Humphrey, and Morrison (*7*) to determine the nutritive value of nonprotein nitrogen for dairy heifers indicated that the total nitrogen of alfalfa hay possesses approximately the same efficiency for growth and milk production as the nitrogen of corn grain plus corn stover. Hart and Humphrey (*4*) showed that milk protein is more efficient than corn or wheat protein when fed with corn stover. These same investigators (*5*) also found that both

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[2] Reference is made by number (italic) to Literature Cited, p. 569.

linseed meal and dried distillers' grains provided a more efficient combination of proteins with corn stover than did corn gluten feed, but in later work (*6*) no differences were found among the three concentrates when clover hay was used as the roughage. Experimental results obtained by Maynard, Miller, and Krauss (*9*) indicated that a ration for milk production based on clover hay furnishes a combination of proteins no more efficient than one based on timothy hay. Morris and Wright (*16*) reported experiments from which they concluded that the nitrogen of beans was superior to that of meat meal or linseed meal for milk production. Each of the feeds in question was fed with a ration of straw, beet pulp, and oats.

In view of the results indicating a rather low quality of protein in alfalfa hay it seemed worth while to obtain further data on this subject and to compare the nutritive value of alfalfa protein with that furnished by clover hay. Since legume hays are rarely fed alone, but are commonly fed in combinations with the cereals or other farm-grown feeds, it would seem that protein studies on these combinations would also be of considerable practical value.

In the series of experiments here reported, nitrogen metabolism studies were conducted with five growing wether lambs. It is believed that the results are, to a certain extent, applicable to cattle as well as to sheep. In these studies alfalfa hay and clover hay have each been fed as the only source of protein and each of these hays has also been fed with corn grain. For each ration the digestibility of the protein, the percentage of protein stored, and the biological value of the protein have been determined and are herein reported.

EXPERIMENTAL METHODS

Five young growing wether lambs have been used in three nitrogen balance experiments. Two lambs were used in the first experiment. They were crossbreds of fairly good type and were thrifty. Three purebred lambs of excellent type were used in the second and third experiments. The lambs were kept in metabolism crates similar in construction to those described by Forbes (*2*). The experimental collection periods were 10 days in length in all the experiments. The intervals between collection periods were also 10 days, which provided ample time to adjust the food intake to a uniform amount that would be consumed daily. Excreta collections were not started until each lamb had been eating the same amount of the ration for at least 6 days.

The feed for each experimental period was weighed out at one time, the daily feed for each lamb being placed in individual paper bags. A representative sample was taken at this time for chemical analysis and served as the basis for computing the nutrients fed. The daily ration for each lamb was fed in 2 equal portions during the first experiment, but the lambs were fed 3 times daily during the second and third experiments. Any unconsumed feed was collected, air-dried, finely ground, and sampled for analysis.

Quantitative collections of urine were made each day, and the urine, together with the washings from the crates, was preserved with toluene in tightly stoppered carboys in a refrigerator room at a temperature of approximately 5° to 10° C. Aliquots from the total volume for each collection period were taken for analysis after the completion of each period.

The daily collections of feces were preserved in airtight containers with acidified alcohol and were stored in the refrigerator. The total quantity for each collection period was then dried at a temperature not exceeding 60° C., weighed, finely ground, and carefully sampled for analysis.

The methods of analysis used were those of the Association of Official Agricultural Chemists (*1*), with the exception of the nitrogen determinations. Nitrogen was determined by the boric acid modification of the Kjeldahl method (*18*).

Several methods may be used in comparing the efficiency of proteins. The writers have used the ordinary cofficient of apparent digestibility, the storage of protein as calculated by the method of McCollum (*8*), and the biological value as computed by the Mitchell method (*10*). The last-named method presumably gives the biological value of a protein or mixture of proteins for both growth and maintenance.

FIRST EXPERIMENT

Two wether lambs were used in this experiment to determine the digestibility, storage of protein, and biological value of the proteins of alfalfa hay, clover hay, and combinations of each with corn protein.

The percentage composition of feeds used is shown in table 1. All the feeds were of excellent quality. The alfalfa hay was second cutting and rather fine-stemmed and leafy. All hay was chopped into lengths ranging from one half to three fourths of an inch. A choice grade of yellow ground corn was used. The composition of each ration as fed is shown in table 2.

TABLE 1.—*Percentage composition of feeds used in the first experiment*

Feed	Dry matter	Ash	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract
Alfalfa hay	93.01	6.57	15.83	2.02	29.41	39.18
Clover hay	90.67	5.59	12.72	2.89	29.03	40.44
Corn, yellow	90.62	1.60	9.15	4.60	1.79	73.48

TABLE 2.—*Percentage composition of experimental rations in the first experiment*

Constituent	Low-nitrogen ration	Alfalfa-hay ration	Clover-hay ration	Alfalfa-hay and corn ration	Clover-hay and corn ration
Straw	40.0				
Alfalfa hay		63.5		47.4	
Clover hay			78.7		59.0
Corn, yellow				27.3	27.3
Cornstarch	23.5	14.6	11.2	6.4	3.4
Cane sugar	23.5	12.5	7.1	5.4	2.3
Cellulose, regenerated	5.0	4.5		8.0	5.0
Corn oil	4.5	2.9	1.0	3.5	1.0
Minerals [a]	3.5	2.0	2.0	2.0	2.0
Total	100	100	100	100	100
Protein content (N×6.25)[b]	1.92	10.09	10.11	10.46	10.35

[a] The mineral mixture contained equal parts of steamed bone meal, ground limestone, and sodium chloride.

[b] These percentages of nitrogen are the averages of analyses of 2 mixes of each ration used.

An attempt was made to feed the lambs a purified diet practically free of nitrogen, but this was unsuccessful and it was necessary to use a low-nitrogen ration containing some wheat straw in addition to the purified ingredients. The nitrogen in the straw was probably not very digestible and this would result in an over-estimation of the metabolic nitrogen. Since a ration entirely free of nitrogen could not be used, however, it was believed that this ration would be fairly satisfactory. Morgen and his coworkers (*15*) used a low-nitrogen ration for sheep, consisting of straw (treated to remove most of the nitrogen), starch, sugar, oil, and minerals. They found that the average excretion of metabolic nitrogen in the feces was 0.51 g for each 100 g of dry matter consumed. Sotola (*20*) fed a similar low-nitrogen ration to sheep and found the average excretion of metabolic nitrogen in the feces to be 0.65 g for each 100 g of dry matter fed. The data obtained in the three experiments reported in the present paper show an average excretion of 0.56 g of metabolic nitrogen for each 100 g of dry matter consumed. It is apparent, therefore, that this value agrees quite closely with those obtained by Morgen and his coworkers (*15*) and Sotola (*20*).

The experimental rations consisted of this low-nitrogen ration and four other rations comparable in character but with the straw and part of the starch and sugar replaced by enough of each feed to furnish approximately 10 percent of protein. In the two rations containing corn, 25 percent of the total protein was supplied by the corn and 75 percent by the hay. All the rations were equalized, as nearly as possible, in regard to dry matter, crude fiber, and total energy content. The rations were all adequate in mineral content. Sufficient vitamins were undoubtedly furnished by all rations, except the low-nitrogen ration. It was believed, however, that this would not be a limiting factor in short balance experiments of this type. The addition of vitamin B supplements would have added nitrogen to the low-nitrogen ration, the value of which would not be known. Regenerated cellulose [3] was used in various amounts to help equalize the "roughage" content of the rations. The lambs were rotated on the different rations according to the customary procedure.

The low-nitrogen ration was fed during the initial and final periods of the experiment. The collection periods were not started until the nitrogen excretion in the urine had apparently reached a constant level as indicated by analyses of the urine for a number of consecutive days. From these collections the losses of nitrogen in the feces and urine were determined and used in estimating, during the periods of protein feeding, the amounts of food nitrogen present in the urine and feces. These data were used in calculating the body's contribution to the feces, or the so-called "metabolic nitrogen", which was expressed in grams of nitrogen per 100 g of dry matter in food eaten. The contribution of the body to the urine was computed in grams of nitrogen per kilogram of body weight. These values were assumed to change in a linear fashion, with respect to time, in the intervening periods between the initial and final low-nitrogen periods.

The results of the first metabolism experiment with the significant intermediate computations are presented in table 3.

[3] Obtained as washed Sylphrap.

TABLE 3.—*Nitrogen metabolism data showing the digestibility and biological value of proteins in the first experiment* [a]

Wether no.	Body weight			Food intake	Dry matter intake	Nitrogen intake	Fecal nitrogen	Estimated metabolic nitrogen	Food nitrogen in feces	Absorbed nitrogen	Nitrogen in urine	Endogenous nitrogen in urine	Food nitrogen in urine	Food nitrogen utilized	Digestion coefficient	Total nitrogen stored	Digestible nitrogen stored	Biological value
	Initial	Final	Average															
	Kilograms	*Kilograms*	*Kilograms*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	
LOW-NITROGEN RATION																		
1	18.18	17.21	17.70	2,066	1,987.3	6.19	10.68	[b] 0.537			7.64	[c] 0.432						
2	25.14	24.75	24.95	4,000	3,853.6	11.52	18.48	[b] .479			11.98	[c] .480						
CLOVER-HAY RATION																		
1	20.36	21.07	20.72	6,000	5,658.0	98.46	43.07	32.25	10.82	87.64	16.98	9.95	7.03	80.61	56	39	69	92
2	27.83	28.14	27.99	8,000	7,523.2	127.52	64.55	33.85	30.70	96.82	33.26	13.44	19.82	77.00	49	23	47	80
ALFALFA-HAY RATION																		
1	21.88	22.79	22.34	6,000	5,683.2	95.52	34.67	33.82	0.85	94.67	26.18	11.84	14.34	80.33	64	36	57	85
2	25.70	26.93	26.32	8,000	7,574.4	130.96	61.29	35.22	26.07	104.89	32.37	12.63	19.74	85.15	53	28	54	81
CLOVER-HAY AND CORN RATION																		
1	25.32	25.87	25.60	6,000	5,551.2	100.62	43.36	36.08	7.28	93.34	28.88	16.13	12.75	80.59	57	28	50	86
2	29.20	29.83	29.52	8,000	7,383.2	130.64	58.46	31.75	26.71	103.93	33.70	14.17	19.53	84.40	55	29	53	81
ALFALFA-HAY AND CORN RATION																		
1	24.02	24.90	24.46	6,000	5,596.8	98.76	30.91	34.98	[d] 0	98.76	26.58	14.19	12.39	86.37	69	42	61	87
2	30.92	31.99	31.46	8,000	7,440.8	135.92	48.38	30.88	17.50	118.42	41.31	15.10	26.21	92.21	64	34	53	78
LOW-NITROGEN RATION																		
1	23.91	21.96	22.94	1,990	1,886.9	7.46	12.77	[b] 0.677			15.60	[c] 0.680						
2	28.90	28.13	28.52	3,566	3,389.1	10.66	13.71	[b] .405			13.68	[c] .480						

[a] Totals are for 10-day experimental periods.

[b] Fecal nitrogen per 100 g of dry matter in feed. These values were used in estimating the metabolic nitrogen in the feces in the experimental periods. The change in the ratio of metabolic nitrogen to dry matter consumed from the first to the last periods was assumed to occur in a linear fashion.

[c] Urinary nitrogen per kilogram of body weight. These values were used in estimating the endogenous nitrogen in the urine in the experimental periods, the same assumption of a linear variation being made as in the case of metabolic nitrogen in the feces.

[d] Estimated metabolic nitrogen greater than fecal nitrogen, therefore it was assumed that no ood nitrogen was present in the feces

The data seem to show that lamb no. 1 was more efficient in utilizing protein than the second lamb. However, greater difficulty was encountered in getting the first lamb to eat the low-nitrogen ration, and it lost considerable weight before it would consume enough food to maintain its body weight. It is possible that when protein feeding was resumed, considerable amounts of the food nitrogen were used for repair and formation of tissue, which resulted in a larger percentage of nitrogen being stored than if the animal had been in a more nearly normal condition. However, Mitchell (*13*) believes that a period of low-nitrogen feeding will exert no appreciable effects upon the utilization of protein in subsequent experimental periods. It would seem, therefore, that the first lamb was simply more efficient in utilizing protein.

Some individual variations can be noted in the apparent digestibility of the protein, the alfalfa being somewhat higher than the clover. The combination of alfalfa and corn had the highest apparent digestibility of the four rations used. The largest storage of protein was obtained with the alfalfa and corn ration; the results with the other three rations were approximately the same. No consistent differences can be noted in the storage of digestible nitrogen.

In regard to the biological values, it is quite evident that no difference in utilization of the absorbed nitrogen has been measured with the different rations. However, the values are quite high and indicate a much greater percentage utilization of nitrogen than has been reported by other investigators for these feeds, especially for alfalfa hay.

SECOND EXPERIMENT

Since no definite conclusions could be drawn from the limited data furnished by the first experiment, the work was repeated with three lambs. The feeds were of approximately the same quality and chemical composition (table 4) as those used in the first experiment, and the same general plan was followed in making up rations and conducting the experiment. This trial, therefore, was simply a duplication of the first one.

TABLE 4.—*Percentage composition of feeds used in the second experiment*

Feed	Dry matter	Ash	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract
Alfalfa hay	93.62	6.38	16.94	2.22	29.28	38.80
Clover hay	94.21	4.99	12.25	2.07	30.41	44.49
Corn, yellow	91.83	1.20	9.82	4.08	2.03	74.70

The results of the metabolism trials are shown in table 5, together with the apparent digestibility, nitrogen storage, and the final biological values of the proteins. These data are in agreement in showing a higher apparent digestibility for the alfalfa protein than for the clover protein. The combination of proteins furnished by clover and corn was more digestible than that of clover hay alone; the alfalfa and corn combination of proteins had the highest apparent digestibility of the four rations fed. When allowance is made for the metabolic fecal nitrogen and the true digestibility of the food nitrogen calculated, the same relative differences in digestibility are still shown. The composition of the rations as they were fed is given in table 6.

TABLE 5.—*Nitrogen metabolism data showing the digestibility and biological value of proteins in the second experiment* [a]

Wether no.	Body weight			Food intake	Dry-matter intake	Nitrogen intake	Fecal nitrogen	Estimated metabolic nitrogen	Food nitrogen in feces	Absorbed nitrogen	Nitrogen in urine	Endogenous nitrogen in urine	Food nitrogen in urine	Food nitrogen utilized	Digestion coefficient	Total nitrogen stored	Digestible nitrogen stored	Biological value
	Initial	Final	Average															
	Kilograms	*Kilograms*	*Kilograms*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	
LOW-NITROGEN RATION																		
3	23.20	21.63	22.42	2,650	2,567.62	6.12	13.54	[b] 0.527			7.58	[c] 0.338						
4	23.53	21.60	22.57	2,326	2,250.76	4.61	12.73	[b] .566			15.53	[c] .688						
5	28.23	26.67	27.45	3,449	3,336.67	8.08	14.80	[b] .444			12.40	[c] .452						
CLOVER-HAY RATION																		
3	24.47	25.00	24.74	8,000	7,656.00	128.56	64.30	43.18	21.12	107.44	34.66	8.58	26.08	81.36	50	23	46	76
4	24.50	25.30	24.90	8,000	7,656.00	128.56	68.45	46.01	22.44	106.12	33.49	15.79	17.70	88.42	47	21	44	83
5	31.17	32.47	31.82	10,000	9,469.00	164.50	84.01	43.18	40.83	123.67	45.23	12.89	32.34	91.33	49	21	44	74
ALFALFA-HAY RATION																		
3	25.27	26.33	25.80	8,000	7,670.40	124.64	55.46	46.10	9.36	115.28	37.89	9.18	28.71	86.57	56	25	45	75
4	25.43	26.73	26.08	8,000	7,670.40	124.64	54.75	48.78	5.97	118.67	38.54	15.10	23.44	93.23	56	25	45	80
5	28.80	30.37	29.59	9,383	9,069.61	142.34	69.28	40.81	28.47	113.87	40.04	12.69	27.35	86.52	51	23	45	76
CLOVER-HAY AND CORN RATION																		
3	30.63	31.17	30.90	8,000	7,468.00	130.08	59.25	50.41	8.84	121.24	28.54	11.53	17.01	104.23	54	33	60	86
4	28.57	29.60	29.09	8,000	7,468.00	130.08	62.26	52.72	9.54	120.54	42.48	13.67	28.81	91.73	52	19	37	76
5	32.57	33.93	33.25	10,000	9,452.00	159.90	67.84	43.67	24.17	135.73	50.30	12.67	37.63	98.10	58	26	45	72

[a] Totals are for 10-day experimental periods.

[b] Fecal nitrogen per 100 g. of dry matter in feed. These values were used in estimating the metabolic nitrogen in the feces in the experimental periods. The changes in the ratio of metabolic nitrogen to dry matter consumed from the first to the last periods was assumed to occur in a linear fashion.

[c] Urinary nitrogen per kilogram of body weight. These values were used in estimating the endogenous nitrogen in the urine in the experimental periods, the same assumption of a linear variation being made as in case of metabolic nitrogen in the feces.

TABLE 5.—*Nitrogen metabolism data showing the digestibility and biological value of proteins in the second experiment*—Continued

ALFALFA-HAY AND CORN RATION

Wether no.	Body weight			Food intake	Dry matter intake	Nitrogen intake	Fecal nitrogen	Estimated metabolic nitrogen	Food nitrogen in feces	Absorbed nitrogen	Nitrogen in urine	Endogenous nitrogen in urine	Food nitrogen in urine	Food nitrogen utilized	Digestion coefficient	Total nitrogen stored	Digestible nitrogen stored	Biological value
	Initial	Final	Average															
	Kilograms	*Kilograms*	*Kilograms*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	
3	27.83	28.90	28.37	8,000	7,652.80	120.96	45.51	48.82	[d] 0	120.96	38.71	10.36	28.35	92.61	62	30	49	77
4	26.97	28.00	27.49	8,000	7,652.80	120.96	49.37	51.35	[d] 0	120.96	40.53	14.43	26.10	94.86	59	26	43	78
5	34.47	36.50	35.49	10,000	9,495.00	154.00	58.56	44.34	14.22	139.78	61.47	12.67	48.80	90.98	62	22	36	65

LOW-NITROGEN RATION

3	29.07	27.70	28.39	2,810	2,697.86	7.66	19.21	[b] 0.712			10.85	[c] 0.382						
4	29.23	27.70	28.47	3,452	3,315.63	9.58	24.57	[b] .741			11.81	[c] .415						
5	36.30	35.13	35.72	4,119	3,952.66	11.62	18.66	[b] .472			11.91	[c] .333						

[b] Fecal nitrogen per 100 g. of dry matter in feed. These values were used in estimating the metabolic nitrogen in the feces in the experimental periods. The change in the ratio of metabolic nitrogen to dry matter consumed from the first to the last periods was assumed to occur in a linear fashion.

[c] Urinary nitrogen per kilogram of body weight. These values were used in estimating the endogenous nitrogen in the urine in the experimental periods, the same assumption of a linear variation being made as in case of metabolic nitrogen in the feces.

[d] Estimated metabolic nitrogen greater than fecal nitrogen, therefore it was assumed that no food nitrogen was present in the feces.

TABLE 6.—*Percentage composition of experimental rations in the second experiment*

Constituent	Low-nitrogen ration	Alfalfa-hay ration	Clover-hay ration	Alfalfa-hay and corn ration	Clover-hay and corn ration
Straw	40.0				
Alfalfa hay		59.1		44.3	
Clover hay			81.7		61.2
Corn, yellow				25.5	25.5
Cornstarch	23.5	15.5	7.7	7.6	2.7
Cane sugar	23.5	15.4	7.6	7.6	2.6
Cellulose, regenerated	5.0	5.0		9.0	4.0
Corn oil	4.5	3.0	1.0	4.0	2.0
Minerals [a]	3.5	2.0	2.0	2.0	2.0
Total	100	100	100	100	100
Protein content (N×6.25) [b]	1.63	9.61	10.16	9.54	10.08

[a] The mineral mixture contained equal parts of steamed bone meal, ground limestone, and sodium chloride.

[b] These percentages of nitrogen are the averages of analyses of 2 mixes of each ration used.

All the lambs showed approximately the same efficiency in storing protein from the alfalfa and clover rations. Some variation was noted in the percentage of protein storage by the individual animals on the other two rations, but the average values are approximately the same.

The biological values are again high, as they average 78 for clover protein, 77 for alfalfa protein, 78 for the clover and corn combination, and 73 for the alfalfa and corn combination. The lower average value for the mixture of alfalfa and corn is due chiefly to the one lower value given by lamb no. 5. The results thus agree quite closely with those of the first experiment, and for convenience of study the results of both experiments have been summarized together for discussion.

DISCUSSION OF FIRST AND SECOND EXPERIMENTS

A summary of the first and second experiments is presented in table 7.

TABLE 7.—*The digestibility and biological value of clover and alfalfa proteins when fed to lambs alone and in combination with corn protein*

Item	Percentage digestibility and biological value when fed to lamb no.—					Average
	1	2	3	4	5	
Clover protein:						
Apparent digestibility	56	49	50	47	49	50
Percentage total nitrogen stored	39	23	23	21	21	25
Percentage digestible nitrogen stored	69	47	46	44	44	50
Biological value	92	80	76	83	74	81
Alfalfa protein:						
Apparent digestibility	64	53	56	56	51	56
Percentage total nitrogen stored	36	28	25	25	23	27
Percentage digestible nitrogen stored	57	54	45	45	45	49
Biological value	85	81	75	80	76	79
Clover and corn protein:						
Apparent digestibility	57	55	54	52	58	55
Percentage total nitrogen stored	28	29	33	19	26	27
Percentage digestible nitrogen stored	50	53	60	37	45	49
Biological value	86	81	86	76	72	80
Alfalfa and corn protein:						
Apparent digestibility	69	64	62	59	62	63
Percentage total nitrogen stored	42	34	30	26	22	31
Percentage digestible nitrogen stored	61	53	49	43	36	48
Biological value	87	78	77	78	65	77

Averaging together the values obtained with the five animals used during the 2 years, it is found that the average coefficients of apparent digestibility are 56 percent for alfalfa protein, 50 percent for clover

protein, 63 percent for the protein in the combination of alfalfa and corn, and 55 percent for the protein in the combination of clover and corn. The application of Student's method for significance of means shows that there is a significant difference in the digestibility of the protein in alfalfa hay and in clover hay. The odds are 188 to 1 in favor of alfalfa protein. Statistical treatment also shows a significant difference between the digestibility of the protein of each hay alone and of the hay fed in combination with corn, the odds in every case being greater than 100 to 1. These same significant differences are shown by the data for true digestibility of the food protein. The alfalfa protein was 89 percent digestible when allowance is made for the metabolic nitrogen in the feces, while the protein of clover hay was 81 percent digestible. The mixture of alfalfa and corn proteins gave a true digestion coefficient of 96 and the clover and corn mixture a coefficient of 89.

The nitrogen intake for each lamb on the different comparable rations was kept at approximately the same level, and it would seem tha nitrogen storage could be used with some reliability as a measure of protein efficiency. The average percentages of total nitrogen stored were 27 percent for alfalfa hay, 25 percent for clover hay, 31 percent for alfalfa hay and corn, and 27 percent for clover hay and corn. The differences between these average values are not very marked and statistical treatment indicates possible significant differences with only two comparisons. Greater storage was obtained from the alfalfa and corn ration than from the alfalfa ration. The odds are 23 to 1 against this difference occurring as a result of chance alone. These odds would indicate a tendency toward significance. A possible significant difference was also measured in protein storage between the alfalfa and corn ration and the clover ration. The odds in this case are 49 to 1 against this difference being due to chance.

The data further indicate no significant differences between the rations when the percentages of digestible nitrogen stored are considered. Some individual variations can be noted between experimental animals, but the final averages are practically identical for the four rations.

In these experiments it was intended to have the protein intake sufficiently high to produce some growth of the animals. This was accomplished as indicated by the live weights of the lambs and by the protein storage which was obtained.

A study of the individual biological values shows some variations, which can probably be attributed chiefly to the individual utilization of protein by each lamb. It is believed, however, that all the values are within the range of animal variation in experiments of this type. The final averages of the biological values show no significant difference between alfalfa protein and clover protein, these values being 79 for alfalfa and 81 for clover. This small difference is due largely to the one high value given by lamb no. 1 for clover protein. Furthermore, the combination of alfalfa hay and corn or clover hay and corn was no more efficient, as measured by the biological value, than these legume hays fed as the only source of protein. The average value for the alfalfa and corn combination was 77, and for the clover and corn combination it was 80.

It is quite obvious that no consistent differences in efficiency of protein utilization have been measured. These biological values for alfalfa hay are much higher than those reported by Sotola (*20, 21*).

Moreover they are considerably higher than those found by Nevens (*17*) in experiments with rates. However, they are in fair agreement with those for alfalfa hay fed to dairy heifers by Hart, Humphrey, and Morrison (*7*) as reported by Mitchell (*14*).

The question naturally arising, after the high biological values were obtained in these experiments, is why are they consistently higher than those reported by most other investigators for alfalfa hay? Since only very few experiments with ruminants have ever shown any measurable differences in efficiency of protein utilization, there is the possibility that the ruminant has the ability to synthesize certain of the essential amino acids as a result of bacterial action in the digestive tract. This possibility has been pointed out at various times in the literature.

Even if sheep do have the ability to synthesize some of the essential amino acids and hence have given high biological values for protein, this does not explain why the values obtained in these experiments are so much higher than those reported by Sotola (*20*, *21*). It is probable that the difference in concentration of protein in the rations would tend to produce a difference in the biological values. Mitchell (*11*) has shown for several proteins that an increase in the level of protein intake results in a lowering of the biological value of the protein. Sotola's (*20*) lambs were fed at a level of approximately 14.2 percent protein, on a dry basis, whereas in the writers' experiments the concentration of protein in the rations was at a level of 10.4 percent. The question remains as to whether the difference in plane of protein intake is entirely responsible for the wide differences in biological value.

There was also a decided difference in the net energy or total digestible nutrient content of the alfalfa-hay ration fed in these investigations and the alfalfa hay as fed by Sotola. The alfalfa ration used in the writer's experiments contained approximately 68 percent of total digestible nutrients, whereas alfalfa hay alone furnishes about 50 percent. It seems quite possible that this difference in net energy and total digestible nutrients may have been a factor in causing the difference in biological values. If fed a ration which is rich in protein and low in net energy, an animal may be unable to use part of the protein for growth, and will of necessity use part of it as a source of energy, thus wasting the nitrogen contained in this portion and lowering the biological value.

THIRD EXPERIMENT

A third experiment was conducted to determine whether low biological values, corresponding to those secured by Sotola, would be obtained if alfalfa hay was given to these lambs as the only feed, as was done in his experiments, and then to find whether high values, such as were previously obtained in the present studies, would be secured if the lambs were changed to an alfalfa ration which supplied plenty of net energy and furnished a lower plane of protein intake. The three wethers used in the second experiment were again employed. The last low-nitrogen period of the previous trial was taken as the first period of this experiment. The lambs were then placed on a ration of alfalfa hay alone with no supplements except common salt. After the period on alfalfa alone, the lambs were fed a ration in which the alfalfa hay was supplemented with starch, sugar, cellulose, corn

oil, and minerals. This ration was just the same as the alfalfa rations fed in the first and second experiments, except that a different sample of alfalfa was used. The protein content of the ration was approximately 10 percent, but the dry-matter and crude-fiber content was about the same as that of alfalfa when fed alone. The lambs were finally placed on the low-nitrogen ration, which had the same composition as those previously fed.

The alfalfa hay used in this trial had the following chemical composition: 8.36 percent moisture, 7.30 percent ash, 17.01 percent protein, 29.86 percent crude fiber, 1.93 percent fat, and 35.54 percent nitrogen-free extract.

The results of the metabolism trials, with the intermediate calculations and final computations, are shown in table 8. Considerable differences were found in the apparent digestibility of alfalfa protein with these two methods of feeding. The protein of alfalfa fed alone had an apparent digestibility of 71 percent, while with the supplemented alfalfa ration the apparent digestibility of the protein was 56 percent. That starch and other nonnitrogenous material may have a depressing effect upon digestibility has been suggested by many writers. However, Mitchell (*12*) has pointed out that the differences in apparent digestibility observed may be explained entirely on the basis of the demonstrated ability of carbonaceous feed to cause an excretion of metabolic nitrogen in the feces in proportion to its dry-matter content. When the values for apparent digestibility in this experiment were corrected for the metabolic nitrogen in the feces there was no difference in the true digestibility. The average true digestibility of alfalfa alone was 92.5 percent, while the supplemented alfalfa ration had a true digestibility of 92.0 percent.

The biological values of the alfalfa proteins obtained with these two rations are particularly interesting. The average biological value of alfalfa protein fed alone was only 50 as compared with 77 for alfalfa fed with starch and sugar in the second experiment with the same lambs. When the lambs were put back on the ration of alfalfa plus starch and sugar, the values averaged 72, agreeing fairly well with those previously obtained with this ration. These data indicate that the low biological values obtained for alfalfa protein when alfalfa hay is given to sheep as the only feed are probably due to the particular method of experimentation and not to any deficiency of the protein.

Biological values of proteins are useful in comparing proteins only under definitely standardized conditions and systems of feeding. In order to secure biological values for the proteins in various feeds that will represent the actual relative nutritive values, it is necessary that the experimental rations furnish the same plane of protein intake. Moreover, they must provide a sufficient supply of digestible nonnitrogenous nutrients so that there will be no wastage of proteins in the rations due to an excessive amount or to an insufficient supply of net energy in other nutrients. If digestible protein is provided in excess of the animal's requirements, or if insufficient net energy is furnished by the nonnitrogenous nutrients to permit full utilization of the protein in the ration, some of it will be deaminized and the nitrogen wasted. Consequently the biological value of the protein will be lowered, not as a result of any deficiency in the quality of the protein, but because of the method of experimentation.

TABLE 8.—*Nitrogen metabolism data showing the digestibility and biological value of proteins in the third experiment* [a]

Wether no.	Body weight			Food intake	Dry-matter intake	Nitrogen intake	Fecal nitrogen	Estimated metabolic nitrogen	Food nitrogen in feces	Absorbed nitrogen	Nitrogen in urine	Endogenous nitrogen in urine	Food nitrogen in urine	Food nitrogen utilized	Digestion coefficient	Total nitrogen stored	Digestible nitrogen stored	Biological value
	Initial	Final	Average															
	Kilograms	*Kilograms*	*Kilograms*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	
LOW-NITROGEN RATION																		
3	29.07	27.70	28.39	2,810	2,697.86	7.66	19.21	[b] 0.712			10.85	[c] 0.382						
4	29.23	27.70	28.47	3,452	3,315.63	9.58	24.57	[b] .741			11.81	[c] .415						
5	36.30	35.13	35.72	4,119	3,952.66	11.62	18.66	[b] .472			11.91	[c] .333						
ALFALFA HAY ALONE																		
3	31.70	31.03	31.37	9,693	8,882.67	263.75	78.50	61.91	16.59	247.16	131.84	12.11	119.73	127.43	70	20	29	52
4	29.50	28.33	28.92	6,930	6,354.06	180.82	51.32	42.32	9.00	171.82	101.07	11.60	89.47	82.35	72	16	22	48
5	35.80	36.27	36.04	10,000	9,164.00	272.10	75.44	45.36	30.08	242.02	134.65	13.05	121.60	120.42	72	23	32	50
ALFALFA-HAY RATION [d]																		
3	32.30	33.67	32.99	10,000	9,404.00	157.30	76.67	64.04	12.63	144.67	46.68	12.87	33.81	110.86	51	22	42	77
4	28.87	29.43	29.15	7,000	6,582.80	110.11	45.43	38.90	6.53	103.58	41.92	11.25	30.67	72.91	59	21	35	70
5	37.23	38.27	37.75	10,000	9,404.00	157.30	64.58	48.62	15.96	141.34	60.53	14.76	45.77	95.57	59	20	35	68
LOW-NITROGEN RATION																		
3	31.07	29.10	30.09	3,000	2,854.80	7.26	19.00	[b] 0.666			11.83	[c] 0.393						
4	28.27	27.50	27.89	3,150	2,997.54	7.62	15.48	[b] .516			10.37	[c] .372						
5	37.53	37.43	37.48	3,900	3,711.24	9.43	20.03	[b] .540			15.72	[c] .419						

[a] Totals are for 10-day experimental periods.

[b] Fecal nitrogen per 100 g of dry matter in feed. These values were used in estimating the metabolic nitrogen in the feces in the experimental periods. The change in the ratio of metabolic nitrogen to dry matter consumed from the first to the last periods was assumed to occur in a linear fashion.

[c] Urinary nitrogen per kilogram of body weight. These values were used in estimating the endogenous nitrogen in the urine in the experimental periods, the same assumption of a linear variation being made as in the case of metabolic nitrogen in the feces.

[d] Ration consisted of 58.8 percent of alfalfa hay, 5 percent of cellulose, 15.6 percent of starch, 15.6 percent of sugar, 3 percent of corn oil, and 2 percent of minerals (equal parts of steamed bone meal, ground limestone, and sodium chloride).

The writers believe that this is the case when alfalfa hay is fed alone with no additional energy supplements. Alfalfa hay fed alone in this third experiment furnished 18.6 percent of protein, on a dry basis, and approximately 52 percent of total digestible nutrients, whereas the supplemented ration contained 10.5 percent of protein and about 68 percent of total digestible nutrients. It seems quite possible that this wide difference may account for the differences in the biological value of alfalfa protein obtained by these two methods of feeding.

In this connection it is of interest to note that Sotola (*19*), in his studies of the biological value for sheep of the protein in alfalfa hay cut at various stages of maturity, pointed out the effect of a difference in plane of protein intake on the biological values of the protein in rations. On an average, the biological value of the protein in the hay cut at the three-fourths to full-bloom stage was 68, as compared with 61 for hay cut at earlier stages of maturity. Sotola concluded that the higher biological values for the later cut hay were "due more to a lower concentration of protein in the ration than to the number of the cutting."

It seems probable that the wide difference in the protein content of alfalfa stems and of alfalfa leaves was the reason Sotola (*21*) secured much higher values for the protein in the stems than in the leaves, when each was used as the only feed for sheep. In these studies Sotola found an average biological value of 64 for the protein in alfalfa stems containing 8.48 to 9.53 percent of protein, on a dry basis, and an average biological value of only 44 for the protein in alfalfa leaves containing 20.34 to 22.20 percent of protein.[4] Similarly, it seems that the wide difference in the plane of protein intake may have been the chief factor in causing the great difference that Sotola observed in the biological values of the protein in corn silage and in alfalfa hay when these feeds were each fed alone to sheep (*20*). In these studies, the average biological value for corn silage, which contained only 5.5 percent of protein on the dry basis, was 94, as compared with 56 for alfalfa hay containing 14.2 percent of protein.

It is obvious that no attempt was made to standardize the rations fed in this third experiment in regard to the protein and energy content. Instead, the object was to find out whether the excessive protein intake and relatively low energy intake as furnished by a ration of alfalfa hay alone would account for the low biological value of alfalfa protein for sheep reported by other investigators. This object was accomplished.

The results of these experiments show that alfalfa hay is probably not deficient in quality of protein for sheep when fed in a balanced ration as regards protein and total digestive nutrients, and that the biological values obtained may depend upon the particular system of feeding and experimentation employed.

These results are especially interesting since it has been assumed that sheep need larger amounts of cystine, the chief sulphur-containing amino acid, than other farm animals. This assumption is based on the fact that wool is high in this amino acid. Some investigations (*3*) have shown a probable deficiency of cystine in alfalfa hay. However, it is known that alfalfa hay is quite high in sulphur. If alfalfa

[4] Since this paper was submitted for publication the detailed report of these experiments appeared in the following publication: Jour. Agr. Research 40: 919-945, 1933. SOTOLA, J. THE NUTRITIVE VALUE OF ALFALFA LEAVES AND STEMS. In this it is stated with reference to the differences in the biological values obtained when alfalfa stems, alfalfa leaves, and entire alfalfa hay were fed separately as the only feeds to lambs, "the variations in values are attributed to the differences in protein concentration of the stem, leaf, and whole-hay rations."

hay is generally deficient in cystine, these results might possibly indicate that this amino acid can be synthesized somewhere in the body, perhaps by the micro-organisms in the digestive tract, from the other sulphur-containing compounds present in the hay.

SUMMARY

In nitrogen metabolism studies with five growing wether lambs, the digestibility, storage, and biological value of the proteins of alfalfa hay and clover hay, and of the proteins in the combination of each hay with corn, have been determined. These rations were equalized in energy content by the addition of cornstarch and canesugar in various amounts.

The average coefficients of apparent digestibility of protein were 50 for clover hay, 56 for alfalfa hay, 55 for the clover and corn combination, and 63 for the combination of alfalfa and corn. These results are statistically significant in showing that the protein of alfalfa is more digestible than that of clover and that the protein of the combination of each hay with corn has a higher digestibility than the protein of either hay when fed as the only source of protein. If allowance is made for the metabolic fecal nitrogen and the true digestibility of the ingested protein calculated, the same relative differences in digestibility were also shown.

No marked differences were observed in the percentage of protein stored from the different rations. However, the difference in the protein storage from the combination of alfalfa and corn and the storage either from the alfalfa-hay or the clover-hay ration, were sufficient to indicate that these differences were possibly significant from a statistical standpoint.

Very little difference was found in the efficiency with which the different proteins were utilized for growth and maintenance, as measured by the biological value. The average of the biological values was 81 for clover protein, 79 for alfalfa protein, 80 for the protein in the combination of clover and corn, and 77 for the protein in the combination of alfalfa and corn.

In an experiment with 3 lambs, alfalfa hay fed with no addition of starch and sugar gave biological values averaging 50, whereas after the addition of starch and sugar to the ration, the biological value was 72. These results indicate the influence which the energy content and the plane of protein intake of the ration may have on the biological value of protein; they also show that values obtained may depend upon the method of experimentation used for their determination.

These experiments show that alfalfa hay is probably not deficient in quality of protein for sheep when fed in a balanced ration.

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